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FOREWORD

The urgency of overcoming agricultural production problems, such as drought and disease, and the dominance of wheat among Canadian agricultural exports, have tended to relegate research on the preservation, storage, and transport of perishable foodstuffs to a position of secondary interest and attention. Of late years the volume of wheat exports has diminished and conversely the exports of meats, fish, fruits and vegetables have increased, so that for the years 1937–39 inclusive, the average annual values were, for the wheat, $107,628,000, and for the perishable foodstuffs, $85,282,000. With increasing difficulty in disposing of wheat surpluses, the trend should be to enlarge our exports of these other products to the maximum possible. Moreover, the production of most perishables being seasonal, preservation is essential to our domestic as well as to our export trade. There is thus every reason to encourage and support these institutions and scientists who have embarked upon a programme of investigations in this field.

Part of the lag in research on food preservation has been due to a lack of the experimental cold storage and other rather expensive laboratory equipment required. While this deficiency is gradually being rectified, it is likely to be a limiting factor for some time yet. It is therefore gratifying to find in the institutions concerned a disposition to pool their resources as far as this may be practicable and necessary to the realization of their common aims. This volume of Collected Papers is part of the fruits of this co-operation.

The Canadian Committee on Storage and Transport of Food grew out of a conference on cold storage held in Ottawa in June, 1934. Its general objective is to promote and co-ordinate Canadian investigations designed to increase the storage life and improve the quality of perishable products which must be stored for domestic consumption or transported to markets in other countries. To deal satisfactorily with the diversified problems coming within its scope, the Committee is organized in four sections, dealing respectively with (1) fruits and vegetables, (2) meats and meat products, (3) fish, and (4) engineering problems. Small panels for the study of taints and refrigerated railway cars have been set up under the section on engineering.

The Committee is sponsored by the National Research Council of Canada, the Dominion Department of Agriculture, and the Fisheries Research Board of Canada. The first of these organizations is studying in its own laboratories the handling and storage of meats, canning problems, and engineering problems. The Department of Agriculture, through its Experimental Farms laboratories, is investigating the processing and storage of fruits and vegetables. The investigation of problems in the preservation and transport of fish is of course a responsibility of the Fisheries Research Board. The Committee has also the co-operation of the Ontario Agricultural College, where a programme of studies on fruit and vegetable storage, designed to utilize the joint facilities of that institution, the University of Toronto, and the Horticultural Experiment Station at Vineland, is under way.

Beginning with 1937, the Committee has issued mimeographed annual reports, consisting of the summaries of the year's progress contributed by members to the annual meetings. This was intended to give to workers in the same field, particularly in other parts of the British Commonwealth, advance information on Canadian studies under way or projected, thus to encourage direct contacts and exchange of information between workers. At the same time the Committee initiated a numbered series of papers, reprints of which can be bound together in volumes to be placed in the libraries of institutions conducting food storage investigations. The number of copies of each volume to be bound is not great enough to make them available to individuals. This first volume is issued with the hope that it may prove a convenient and useful reference both to members of our own Committee and to workers in other places.

ROBERT NEWTON, CHAIRMAN,
Canadian Committee on Storage and Transport of Food.

February 5, 1941.

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Table of Contents

Table of Contents, continued

Table of Contents, continued

Table of Contents, continued

Freezing and Frozen Storage of Poultry

By Dr W. H Cook and Dr. L. Sair

INTRODUCTION

The seasonal production of poultry in Canada, combined with the need for transport over relatively long distances to both domestic and export markets, has resulted in a considerable proportion of this product being handled in the frozen state Canadian practice has also been influenced by the growing tendency in the United States to store and transport poultry in the frozen condition In consequence, most of the investigational work on this continent has been directed toward improvement, or solution of difficulties encountered, in the frozen storage of poultry

During recent years the processing and storage of poultry has become fairly well standardized wherever adequate facilities are available After plucking, the birds are pre-cooled as quickly as possible by hanging them on racks in a room at 30° to 32 F, and they are usually left at this temperature for 24 hours before grading and packing for freezing Some operators cool the birds even more rapidly by immersing them in cold water for a short period before hanging them in the cooler The poultry, packed in boxes containing twelve birds are then " sharp-frozen " at temperatures between 10° F. and 0° F. for a period of three days or longer, and are subsequently stored at temperatures of from 0°F to + 10 F

Within the past few years a few new practices have been introduced. There is a decided tendency toward the use of lower temperatures for frozen storage. In the United States storage temperatures of — 10° F. are not uncommon, and temperatures of - 20ᶜ F. are being used for poultry storage in certain warehouses. These low temperatures have come into use primarily for reducing surface desiccation or freezer-burn, which becomes evident during the storage period, but certain other advantages are also claimed by commercial operators. Another departure from standard practice has been introduced recently by the marketing of full-dressed or drawn poultry. Such poultry are eviscerated and prepared for the oven before being frozen. No standard practice has yet been developed for preparing this product. Some precool the birds thoroughly, as before, and grade them before they pass to the eviscerating table. Others feel that the birds need only be cooled enough to permit clean workmanlike evisceration, after which they are usually frozen immediately, usually by a quick-freezing procedure. This quick-freezing process may be accomplished in several ways, but the object is the same, namely, to pass the poultry through the freezing zone in from 1 to 2 hours, instead of from 1 to 3 days, which is approximately the time required to freeze the boxed product at modern " sharp-freezer " temperatures.

Investigations have been undertaken in the cold storage laboratories of the National Research Council of Canada, to determine the value of certain phases of the older standard practices and the newer modifications. The status of these investigations is reviewed briefly in the following sections of this article.

Precooling of Poultry.

Current practice on this continent is to reduce the temperature of product from that of animal to near the freezing point of water as quickly as possible. This practice not only promotes efficiency, where the product is being processed on a large scale, but is also claimed to reduce the shrinkage, and the microbal growth on the surface. The older idea of removing the " animal heat " slowly is rapidly passing into oblivion, although slow cooling may be beneficial by allowing certain desirable post-mortem changes in the muscles to occur.

The precooling of poultry was first studied from. the purely physical standpoint, in order to determine how quickly this process could be accomplished by the methods ordinarily employed. The principal factors taken into account were : the temperature difference between the product and the air in the cooling room ; and the weight

of the bird. Other factors, such as the shape of the bird, and the quantity and disposition of fat, also affect the cooling rate, but as these quantities cannot be readily estimated, their quantitative influence was not determined. The birds were precooled in a room at 32 F., but as the temperature of the product on receipt at this room was variable, a considerable range of temperature differentials and cooling times was obtained. A large number of birds were therefore studied, and the results used to construct a curve relating the time required for cooling to the initial temperature difference and the weight of the birds. It was then found that about half of the observed variability in the cooling time could be accounted for by the variable initial temperature differences, and the weights of the birds

The equation of the cooling curves in terms of the observed quantities was ·

$$T = 5.00 + 6.23 \log (t_r - t_a) + 1.156 \, W.$$

where T = time in hours to cool the product to 2 F. above the room temperature (in this case 34 F.).

/ = product temperature on receipt at cooling room.

t_a = air temperature in precooling room

W = weight of bird in pounds.

The logarithm of the temperature differences was used, since it was found repeatedly that the cooling time required was a linear function of the temperature difference. The above equation is therefore applicable to any room or product temperature between blood temperature and the freezing point. An equation containing a second-degree term for weight was also tested, but, on the whole, this did not give a better fit than the linear equation over the weight range used. The mean cooling time and the residual variance unaccounted for by the above equation, expressed as the standard deviation, was 6.9 ± 1.8 hours for birds having a mean weight of 4.4 lb.

Placing birds, having a mean weight of 3.6 lb in water at 32° F. for two hours after processing, and then hanging them in air at the same temperature, cooled them to 34° F. in 2.9 ± 0.7 hours. Birds cooled in this way showed an increase in weight of extremely variable magnitude, the average gain and standard deviation being 2.2 per cent. $\pm$ 3.1 per cent. The air-cooled birds, on the other hand, showed a loss or shrinkage, of 0.23 per cent. + 0.11 per cent. No difference could be detected between the surface appearance or bloom of the birds cooled by the two methods during subsequent storage in the frozen condition.

FREEZING OF POULTRY.

The time required to freeze boxed poultry depends on a number of factors, including the air temperature, air movement, method of piling, insulating qualities of the container and the net weight of the box, etc. Repeated experiments indicate that such factors as air movement, method of stacking, and the insulating qualities of the box, all of which are difficult to standardize rigidly in the ordinary commercial packs, have about as much effect in determining the freezing rate as the variations in net weight usually met in commercial practice. These results will not be considered in detail, but the time required for the temperature at the centre of the birds to pass from 32° F. to 25° F. is given in Table I. This temperature interval was taken to represent the freezing zone. The reported values are the average of tests made on five boxes of different net weights, the mean net weight of the group being 49 lb. It is evident that about 50 hours will be required to freeze the product at freezer temperatures of 0° F. to −10° F.

At present very little poultry is quick-frozen in Canada, but this process is being practised to a considerable extent in the United States. The packers and consumers alike acclaim the superiority of this product, but there is comparatively little scientific information on this subject, and some of it is conflicting. Certain investigators (5) claim that the quick-frozen material is superior in flavour, while other results (9) suggest that the rate of freezing has little effect on the quality. It seems probable that pre-selection of the product to be quick-frozen, and the perfection of the storage, transport, and distributing chain now used for handling the quick-frozen product may contribute more to the final quality than the quick-freezing process.

Several advantages can be claimed for quick-freezing from the scientific standpoint, but only one of these will be dealt with here. It is a well established fact that when beef or fish are frozen by the usual " slow " method, large ice crystals form in the muscular tissue. When the product is thawed the water contained in these crystals is not retained entirely by the tissue, and " weeping " or " drip " results. This drip causes a loss of quality as well as shrinkage, since the exuded fluid contains some of the flavouring and nutritive constituents. By analogy it has apparently been assumed that poultry meat would also drip after slow freezing, although slow-frozen mutton and pork do not appear to drip to any extent on thawing (3). Mandeville (8) expresses the opinion that the advantages

of quick-freezing may not be attributable to the prevention of drip, but some operators feel that slow-frozen poultry may suffer a loss in quality from the formation of drip on thawing   Otherwise the authors are unaware of any definite information on this point

Preliminary experiments in this field indicate that frozen poultry did drip slightly on being thawed, a value of about 2 per cent. being obtained with slow-frozen, and 1 per cent with quick-frozen material   It is obvious that losses of this magnitude cannot be measured with sufficient accuracy to establish the relation between freezing rate and drip   By grinding the poultry meat the quantity of drip was increased, and this made it possible to determine the effect of freezing rates on drip formation   The method consisted essentially of grinding a sufficient quantity of meat, drawing individual samples of about 100 grams, weighing accurately, and then freezing in small metal dishes at the regular rate. After thawing the free moisture was absorbed with blotting paper and the drip computed from the loss in weight, after subtracting the percentage loss obtained in unfrozen control samples This is essentially the method used by Reay (10) in studying the drip from fish.

This method gave higher values for the loss of weight by drip, and it was therefore possible to determine the effect of different rates of freezing   It was also found that the quantity of drip depended on the precooling time prior to grinding and freezing   When the birds were not precooled or allowed to hang less than 12 hours in air at 32 F , much more drip was obtained than when the birds were allowed to hang 24 hours or more before freezing Reference to the equations given in an earlier section shows that the birds would be cooled to within a few degrees of room temperature in less than 12 hours, so that this effect could not be attributed to inadequate cooling   It was therefore concluded that certain post-mortem changes in the muscle must be allowed to take place before it is frozen, if the quantity of drip is to be reduced to a minimum.

Typical results obtained in these experiments are given in Table II, together with the freezing rates and precooling treatments used. Where the poultry are allowed to hang for an adequate period before freezing it is evident that the drip decreases as the freezing rate increases. The greatest change in the slope of this curve, relating freezing rate and drip, occurred at a rate requiring about 1½ hours to pass from 32° F. to 23° F. Where the poultry meat was frozen within three hours of slaughter there appears to be no advantage in quick-freezing, the quantity of drip being relatively constant at all freezing rates, and being equal to,

or greater than, the maximum quantity obtained from birds that had hung overnight. The relatively constant quantity of drip obtained at all freezing rates from these freshly killed birds is probably the result of two opposing tendencies, slow freezing on the one hand, tending to increase the quantity of drip, but, on the other, allowing time for the post-mortem changes which reduce the drip to take place.

It is concluded from these results that since the quantity of drip exuding from the whole bird on thawing is extremely small, its loss would probably have little effect on the final quality. If, however, this small quantity, or the changes causing its formation, is sufficient to cause deterioration in quality, then hanging for a sufficient period to allow the post-mortem changes to occur must precede freezing. The results also indicate that the freezing rate must be capable of passing the material through the temperature interval, 32° F. to 23° F., within 1¼ hours if the drip is to be reduced to significantly less than that obtained by sharp-freezing in the ordinary way.

The nature of the post-mortem changes which reduce drip formation is as yet unknown, but is under investigation. It is also felt that the claimed superiority of quick-frozen poultry must be explained otherwise than by the effect of the freezing process on crystal size and drip formation, and several possibilities are being studied.

FROZEN STORAGE OF POULTRY.

At the freezer temperatures (+10° F. or lower) ordinarily employed in North America for the storage of poultry, the first evidence of deterioration comes from a loss of bloom accompanied, or followed, by the appearance on the skin of circular or irregular areas of lighter or darker colour. Such poultry are referred to as pock-marked or freezer-burnt, and owing to their appearance are difficult to market to advantage.

When investigations were undertaken on this subject it appeared that the primary cause of the condition was surface desiccation, although other chemical and physical changes in the skin and flesh were offered as explanations (12). Repeated experiments of a preliminary nature showed that pock-marking or freezer-burn never occurred in products stored over ice, i.e., at 100 per cent. relative humidity on an ice basis. This indicated that if other physical or chemical changes took place they were secondary, the primary factor causing freezer burn being a drying of the skin.

A quantitative study of this subject was then under-

taken by storing poultry at temperatures of 7.5° F. and
—7.5° F. over solutions of calcium chloride at different
vapour pressures (*1*, *7*). The corresponding relative
humidities were computed, taking the vapour pressure of
ice at each temperature as 100 per cent. As the calcium
chloride solutions suffered some dilution from the water
coming from the product during the storage period, the
mean relative humidity to which the poultry were exposed
is probably somewhat higher than the initial values
reported in Table III.

This table shows the time required to produce pock-
marking or freezer-burn at the temperatures and relative
humidities studied, and also the estimated percentage of
the breast area affected after 19 months' storage. At the
conclusion of the experiments the moisture content of the
skin was determined. These results were somewhat
irregular, owing to the variable fat content of the skin,
and the difficulties of obtaining and sampling such small
quantities of frozen material. They are not reported, but
show a gradual decrease in moisture content from about
49 per cent. in the container at 100 per cent. relative
humidity to 26 per cent. in the container at 75 per cent.
relative humidity.

The results in Table III show that the product suffers
commercial deterioration in quality from surface drying
at 85 per cent. relative humidity, in about six months at
—7.5° F., and in less than three months at +7.5° F.
Storage periods of six months are quite common com-
mercially, and a relative humidity of 85 per cent. is higher
than that existing in most freezers. Since the drying power
of the air is decreased by lowering the temperature, even
when the relative humidity remains the same, it is natural
that the freezer-burn should take longer to appear, and
be less severe, at —7.5° F. than at 7.5° F. The birds
stored over ice (100 per cent. R.H.) at both temperatures
had excellent bloom and there was no evidence of surface
drying when the experiments were terminated

Two methods are now in use commercially for reducing
deterioration by freezer-burn. The first of these has been
the replacement of the usual parchment paper box liner
with materials less permeable to water vapour, such as
waxed paper, moisture-proof cellophane, etc. Measurements
of the relative humidity within poultry boxes lined with
such materials indicate that they are superior to parchment.
Nevertheless, the relative humidity is frequently too low
to prevent freezer-burn over a six-month storage period at
a temperature of 7.5° F. This result is attributed to the
variable condition of the folds of the liner which it is

commercially impracticable to seal. Some consideration has been given to the use of waterproofed corrugated cartons of such a design that they can be easily sealed.

The second method used for preventing freezer-burn has been to reduce the drying power of the air by lowering the temperature. This has led to the use of storage temperatures of $-10°$ F., and even $-20°$ F. for poultry in the United States. Although these low temperatures may confer additional advantages, by retarding other detrimental changes, the existence of such changes at the usual freezer temperatures and storage periods has not been definitely established. It appears therefore that reducing the temperature is a costly method of reducing the drying power of the air to a point where it will reduce, or prevent, deterioration from surface drying.

The drying power of the air is determined both by the temperature and the relative humidity in accordance with the equation (1) $\dfrac{dw}{de} = k(p_w - p_a)$, where $\dfrac{dw}{de}$ is the rate of evaporation, p_w is the vapour pressure of the evaporating water, p_a that of the water vapour in the air, and k a constant, dependent in this case on the velocity and flow characteristics of the air. It is therefore evident that if p_a could be increased the storage life of poultry might be increased considerably without resorting to sub-zero temperatures. In most spaces kept at temperatures below the freezing point the temperature of the cooling grids is of the order of $10°$ F. lower than that of the ambient air. The dew point of the air in such a space when empty will therefore be about $10°$ F. below the air temperature, and p_a will then be about 65 per cent of saturation or less. The vapour pressure of the product will be about 0.95 per cent. of saturation or more (2), and when it is placed in the room the air will assume a relative humidity somewhere between these two extremes. Since the poultry boxes are always lined in some way, the relative humidity of the air within the box will always be somewhat higher than that of the air circulating over the coils. The humidity gradient across the box, however, will depend not only on the efficiency of the liner, but also on the relative humidity of the air in the store.

One method for increasing the relative humidity of the air in freezers has been suggested by Huntsman (6). This involves jacketing the space, between the cooling grids and the storage space, with a moisture-impermeable but heat-transmitting material, such as sheet metal. Since the air surrounding the product does not come in contact with

the cooling coils, the dehydrating effect of the latter is avoided, and the relative humidity approaches that corresponding to the vapour pressure of the product. Although this may be satisfactory for products that are not packaged for storage, it is apparently not satisfactory for boxed poultry. This method was tested by placing the packaged product in tight metal tanks and storing them for a year at -| 7.5° F. At the end of the period the product was badly freezer-burnt. It seems probable that the absorption of moisture by the wooden boxes (II) exerted a drying influence on the atmosphere. If these deductions are correct, surface desiccation of the product cannot be avoided entirely by preventing the transfer of moisture from the product to the cooling coils, and must be supplemented by supplying moisture to the air from some other source than the product.

Since the transfer of heat from the air to the coil inevitably involves, at high relative humidities, the transfer of moisture in the same direction, it is obvious that the mere addition of water vapour will not accomplish the desired result. Such additions of moisture will merely coat the cooling grids with frost much faster, with little permanent compensating advantage from an increased relative humidity. The method of humidification devised in the National Research Laboratories involves the circulation of brine or other aqueous solution having a freezing point lower than the coil temperature, over the cooling surfaces to prevent the formation of ice. This solution is collected by suitable drip trays and returned to a small tank for recirculation by the pump. Since such a solution will naturally have a lower vapour pressure than ice, at the temperature of the coil surface, it follows that no humidification is accomplished at this stage. A small regulated portion of the solution in the tank, or of that returning from the drip trays, is therefore passed through an electrical heater, where it is boiled and the resultant water vapour discharged into the space. Slow circulation through the heater insures that only the minimum amount of liquid is heated, while preventing troublesome accumulation of the non-aqueous phase at this point. The moisture cycle by this system is therefore : condensation on the wet cooling surfaces, return to the tank or heater by the aqueous solution ; and hence from the heater to the air in the store by evaporation.

Using this system it has been found possible to maintain a relative humidity of from 90 to 95 per cent. (measured by a special dew point apparatus) for several weeks at |-8° F. when the temperature of the brine in the cooling grids had

a mean temperature of — 1° F. All need for defrosting the coils is eliminated, and an increased heat transfer efficiency is obtained since the formation of frost is avoided. The concentration in the tank only requires periodic adjustment, depending on the frequency with which the doors are opened. By installing a heat exchanger between the hot and cold liquids leaving and entering the heater and suitable insulation on all hot lines, it has been possible to obtain efficiencies such that the latent heat of the evaporated water accounted for 65 per cent. of the total heat input.

Nevertheless, it is obvious that the entire heat input is added to the refrigeration load wherever the entire apparatus is installed within the storage space. This is partly compensated by the better heat transfer from the ice-free coils, and the fact that the heat of fusion of this ice is saved. It is therefore of interest to compare the value of this method of reducing the drying power with that accomplished by reducing the temperature. Tests are still being made on this system of humidification, and the following results are to be regarded as tentative. It has been found possible to maintain a relative humidity of from 90 to 95 per cent. when the heat input was 10 per cent. or less of the heat gaining entrance through the walls. If the drying power of the air were to be reduced to the same value by lowering the temperature, the room would have to be cooled to below — 10° F. at 65 per cent. relative humidity. This increased temperature difference across the walls of the store would require at least 20 per cent. more refrigeration for maintenance, even if the reduced capacity of the compressors is neglected, or over twice as much as the extra refrigeration required for humidification by the system described.

TABLE I.

Time required to freeze boxed poultry.

Air temperature ° F.	Time required for temperature in centre of bird to pass from 32° F. to 25° F.
	Hour
22.2	275
9.0	66.0
— 3.5	49.5
— 18.9	31.0
—35 0	19.6

TABLE II.

*The effect of promptness and rate of freezing on the formation
of drip in chicken meat.*

Rate of freezing hours (32° F. to 23° F.)	Frozen within three hours of killing	Frozen after precooling for 24 hours at 32° F.
	Net drip	Net drip
	per cent.	per cent
1.0	9 7	2.4
1.3	8 6	4 2
2 5	7 2	5.3
8.0		6 9
18 0	8 7	7.3

TABLE III.

*Surface drying (freezer-burn) in relation to storage temperature
and humidity*

Storage conditions		Time for definite surface drying	Estimated proportion of skin area affected after 83 weeks
Temp F.	Relative humidity (initially)		
	per cent	weeks	per cent.
− 7 5	70	13	5–10
,,	75	8 -9	5–10
,,	80	10–11	5 -10
,,	85	22	5 10
,,	90	37	5
,,	95	None in 83	None*
,,	100	None in 83	None*
+ 7.5	70	8	40 -60
,,	75	9	10- 20
,,	80	11	20 25
,,	85	9 10	15- 25
,,	90	23	5 -10
,,	95	37	Slight*
,,	100	None in 83	None*

* The lots had not suffered commercial deterioration from
surface drying after 83 weeks storage

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STUDIES OF THE TOMATO IN RELATION TO ITS STORAGE

I. A SURVEY OF THE EFFECT OF MATURITY AND SEASON UPON THE RESPIRATION OF GREENHOUSE FRUITS AT 12.5° C.[1]

By E. J. M. Walford[2]

Abstract

Tomatoes were grown in the greenhouse at different seasons of the year, individual fruits were picked at various stages of maturity and continuous records of their respiration obtained at 12.5° C. It was found that the fruits of the late spring and summer went through the customary series of extensive changes in respiration rate as they ripened at the low temperature, and exhibited the lack of durability normal to this fruit. In contrast to this, the fruits of the late autumn, winter and early spring, if picked before the external appearance of red pigment, passed into a stable state in which ripening proceeded with but little change in respiration rate and with greatly enhanced duration of life at 12 5° C.

The tomato fruit has been investigated with a view to storage much less than the dessert fruits, doubtless because so large a portion of this crop is processed by canners. Nevertheless important quantities, and especially the greenhouse crops, are consumed fresh, and the storage properties of this fruit are of importance in its marketing and transportation. In the future we may anticipate that, as with other fruits, the problems of storage will come to influence even culture and harvesting practice.

Experience shows that the tomato's natural lack of durability is not easily overcome by low temperature. The fruit tends to be intolerant of the severe retardation of its ripening processes by temperatures near 0° C., and to break down rapidly upon removal to higher temperatures after quite short exposures to low temperature (9). The consequence is that moderate, compromise temperatures in the vicinity of 10° to 12° C. are most commonly advocated for field-grown tomatoes in temperate regions (1, 9), though somewhat lower temperatures appear to be permissible for tomatoes grown in the tropics (8). Moderate temperatures usually mean short-term storage for the purpose of increasing the flexibility of marketing, rather than for preservation beyond the season.

The outlook for long-term storage is not very encouraging. Yet it is known from farm experience that late tomatoes removed to a suitably cool place in the autumn may be held, ripening gradually and yielding a supply of marketable fruit, well on into the winter. This suggests that the seasonal factors operative during the growth of the fruit may be effective in influencing durability. There is reason to believe also that growth conditions may be so adjusted as to diminish the intolerance of the tomato to low temperature.

For instance, Barker (1) found that temperatures below 15.5° C. were injurious to English hothouse tomatoes, whilst Wardlaw and McGuire (8) showed that their tropical fruit was better preserved at about 5° C. Kidd and West (6) later discovered that English summer-grown fruit tends to behave in respect of temperature in the manner of the tropical tomatoes, whereas English autumn-grown fruit gave results such as those obtained by Barker. Evidently there is something to be learned about the influence of season upon the properties of the fruit that concern its preservation in the living state.

Consequently, when it was decided in 1934 to begin the study of tomatoes in relation to their storage, it was felt that one of the most useful additions to our knowledge would be attained by systematic examination of the influence of season, and of maturity at the time of picking, upon pertinent aspects of the physiology of the fruit held at some appropriate storage temperature. The temperature adopted was 12.5° C. It was planned to combine with this survey such bulk storage tests as the results of the physiological enquiry might demand.

This project in its two aspects of physiological survey and storage testing is still in progress. The physiological work done by the writer has chiefly taken the form of tracing the respiration of the fruit throughout its life at the storage temperature. This choice was made because the record of respiration is the most generally useful single index of the gradually changing physiological state of the fruit. To be fully significant such a study demands the treatment of single fruits, otherwise individual peculiarities are obscured by statistical effects. This would defeat one main purpose of the enquiry, which is to detect and characterize at least the more frequent physiological types occurring amongst the fruits at all seasons of the year in correlation with the cyclic variation of the conditions of growth.

The present paper gives an account of the respiration at 12.5° C. of individual fruits picked at various stages of maturity from plants grown in the greenhouse at different seasons during the first year of the investigation. This systematic survey was continued for another two years with confirmatory results, so that a much larger number of fruits have actually been under observation than are reported upon in detail in this paper. It happens that the data of the first year, with slight supplementation, illustrate sufficiently the course of respiration in the fruits, and mere recapitulation of substantially similar results could serve no purpose. The individual records will be examined and compared in some detail, since they form an index to the physiological changes occurring in fruits stored at 12.5° C. They are also the criteria by which we distinguish two types of fruit, the distribution of which has been found to be correlated with the seasonal factor. These types will be constantly before us in later papers of this series, which will embody the results of attempts to modify the distribution of the types through the manipulation of growth conditions, the further characterization of the types, and the history in bulk storage of fruits produced by various methods.

Experimental Procedure

Propagation of the Plants and Growth of the Fruit

Tomato plants of Grand Rapids variety were grown in the greenhouse from seed obtained from a commercial seed house. A standard procedure for germination and transplanting was devised, and the soil kept as uniform as possible throughout. The bench soil was a light clay loam with which was incorporated well rotted manure (30 tons per acre) and a 0-12-12 fertilizer (1500 lb. per acre). When the third truss of fruit was forming, weekly side dressings of nitrate of soda were applied (150 to 200 lb. per acre). The plants were set out with 18-inch centres and grown to a single stem. They were supported and allowed to produce seven trusses of fruit.

The mean temperature of the greenhouse varied with the season, but abrupt or violent fluctuation was avoided. The light periodicity normal to the season was not modified in any of the experiments dealt with in this paper. Pests were not serious, but some fumigation was unavoidable and cyanide was employed at least two weeks before picking the fruit. There was no detrimental effect and we are satisfied that any physiological disturbance occasioned at the time of fumigation was well past before respiration experiments began.

Pollination was assisted by tapping the flowers. The setting of the fruit was identified by the ready separation of the corolla from the receptacle and the date of setting was recorded on a tag attached to each young fruit.

Classification of the Fruits

The age of each fruit was known but the growth and physiological development of the individuals vary, so that fruits of the same chronological age are not necessarily in identical physiological states. It was essential, therefore, to consider other indices of development in choosing the experimental fruits. Consequently, a series of classes was established, within the limits of each of which the fruits would be at least roughly comparable physiologically. This classification is given in Table I. The subsequent physiological test confirmed the assignment of fruits to their place in this classification in all but exceptional cases.

Measurement of the Respiratory Carbon Dioxide

Immediately after picking, the fruits were removed to a room at 12.5° C. the calyces were carefully taken off, the fruits weighed, the calyx scars waxed and the fruits re-weighed. A full description of each fruit was recorded. The fruits were then placed singly in glass respiration chambers consisting of two hemispheres sealed together and provided with tubulatures. The air flow was started, and zero time of the respiration record was taken the following morning, approximately 15 hours later. The air was passed through large towers of soda lime and humidified in 7% potash solution before being led into the respiration chambers. From the chambers each stream passed to a Pettenkofer absorption tube containing barium hydroxide. The Pettenkofer tubes were changed periodically and the residual baryta titrated. The

TABLE I

PHYSIOLOGICAL CLASSIFICATION OF TOMATO FRUITS

Physiological stage	Symbol indicating physiological state on respiration records, tables, etc.	Description of the fruit
Early growing-green	I	Immature, growing, hard, dark green and small in size for the variety. Chronological age up to 32 days.
Late growing-green	II	Maturing, hard, green, medium size for the variety. Chronologically 32 to 42 days.
Mature-green	III	Maximum growth, firm, light-green color.
Yellowing	IV	Yellow color appearing on the base, remainder light green.
Yellow-orange	V	Orange-red base and yellow-green calyx.
Three-quarters red	VI	Evenly orange-red.
Full red	VII	Red.

successive absorption periods were of 24 hours duration in the experiments on fruits from Populations I, II and III, and of 12 hours in the others. Mean rates for each interval were computed in terms of cc. of CO_2 per 10 kg. of fruit per hour. These determinations are represented graphically in Figs. 1 to 35.

As the experiment proceeded, notes were kept of the maturation and senescent changes in the fruit. Where the fruit cannot be handled or destroyed, the only available index of ripeness is external color, so that the correlation of respiration rate with ripeness is essentially a correlation with color. Each graph is provided with a chart which indicates the progress of external color change along the time scale of the respiration record.

The Experimental Materials

The fruits used in the survey experiments of the first year were derived from four plantings of tomatoes which are referred to as Populations I to IV. They were picked at various stages of maturity, but owing to limitations of apparatus, each population could not be represented by fruits of every stage. The character of the fruits is indicated in Table II, in which they are assigned to their parent population and classified according to the scheme previously described.

Figs. 37 and 38 summarize the chronological distribution of the periods of growth and of picking for each population and relate them to the factors of light and temperature.

Populations I and II were summer-grown, and since the records indicate that the fruits of the two populations constitute a single series, they are

TABLE II

NUMBERS OF FRUITS OF POPULATIONS I TO IV, IN VARIOUS PHYSIOLOGICAL STAGES

Population	Early growing-green	Late growing-green	Mature-green	Yellowing	Yellow-orange	Three-quarters red	Full red
	I	II	III	IV	V	VI	VII
I			2	2	2	2	3
II	4	3					
III			3	2		3	2
IV	3				2		2

treated together. Population III was seeded in July and the fruits examined were autumn-grown. Certain of these fruits provided respiration records that form a striking contrast with corresponding summer-grown fruit. The implication of the respiration studies is that the vast majority of autumn-winter-grown fruits when isolated at appropriate stages of maturity and kept individually at 12.5° C. are in a different physiological state from the summer-grown fruit. The difference is interesting from the point of view of storage because it involves a great extension of life at the temperature employed. The plants of Population IV were started in winter, but fruited in spring and represent the spring population.

Experimental Results

Fruits of Populations I and II

The respiration records of eleven fruits from Population I are given in graphic form in Figs. 1 to 11. Before turning to the data, comment must be made on certain fluctuations of great amplitude occurring in some of the earliest records obtained. These fluctuations are quite distinct from the normal fluctuations found in every respiration record. Their origin was traced to stoppage of the air stream by deposition of barium carbonate in the narrow delivery tubes with which the Pettenkofer tubes were originally furnished. Replacement of the delivery tubes removed the difficulty. Points known to be affected by this circumstance are neglected in drawing the lines.

Records 1 and 2 are of fruit classified as Stage III. mature-green at the time of picking. Respiration rises steadily to a maximum of 100 to 110 cc. CO_2 in 12 days and then drops to about 70 cc., completing the rise and fall in about 27 days. After a few days of steady rate tissue breakdown occurs, carbon dioxide output rises, and the determinations end.

The ripening color changes commence almost immediately after the beginning of the record, so we must conclude that the fruits were in the last stages of mature greenness at the time of picking. The fruits change from yellow-green to orange during the rise of respiration and the transition to red is
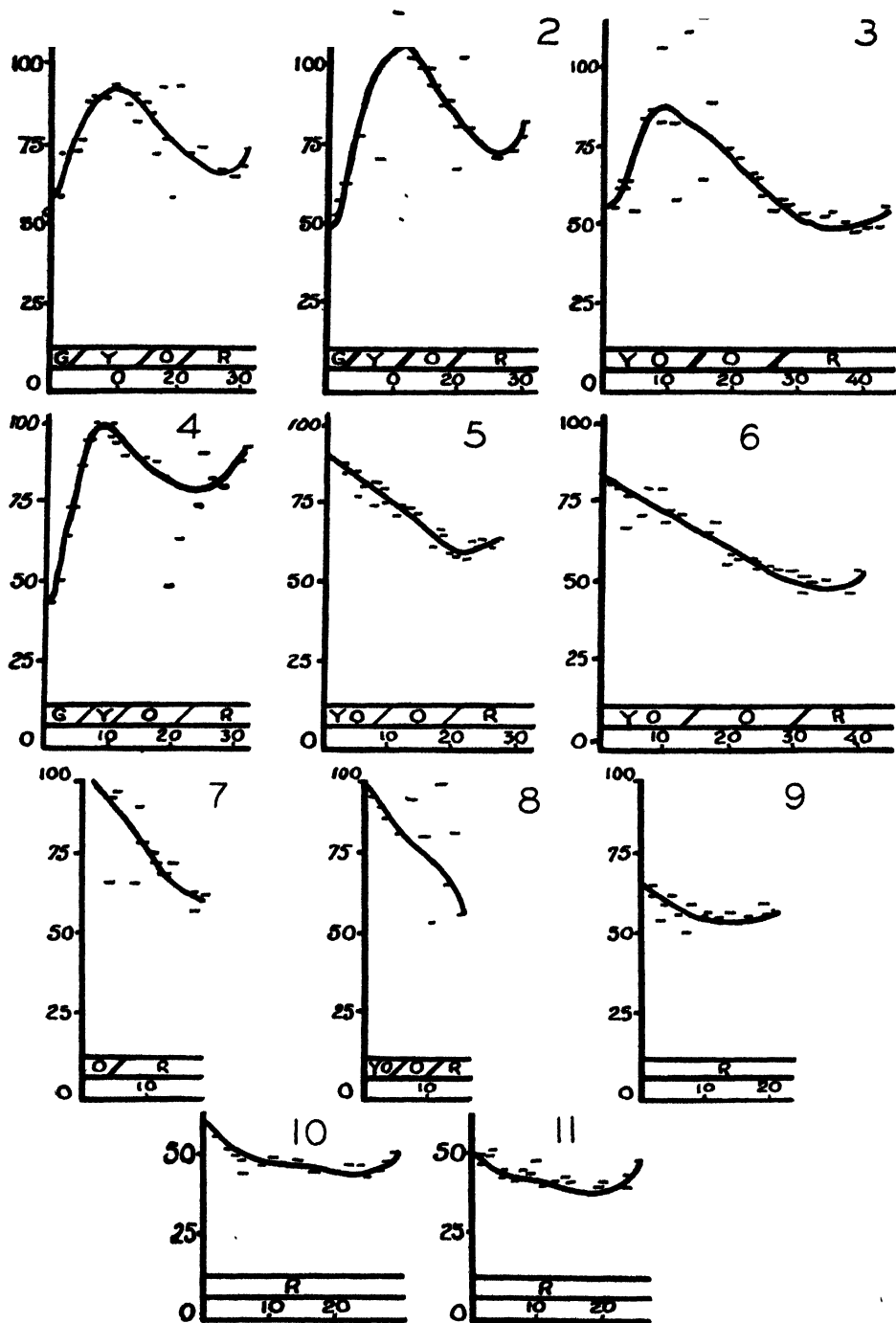
FIGS. 1–11.   *The respiratory drift in storage (at 12.5° C.) of fruits of Population I.
Vertical axis indicates cc. CO₂ per 10 kg. per hr.; horizontal axis, time in days and color
changes in the fruit (G = green, Y = yellowing YO = yellow-orange, O = orange, R = red).*

accompanied by falling respiration rates. The correlation of the visible ripening color changes with a rise and fall of respiration confirms Gustafson (4), who found that ripening tomatoes pass through these respiratory phases. This phenomenon appears to be an almost general property of senescent plant tissues. The "senescent rise of respiration" was long ago observed by Blackman (2) in starving leaves, and more recently by Blackman and Parija (3), Kidd and West (5, 6, 7) and others, in isolated fruits such as apples and pears. It must be regarded as the conventional mode of behavior of ripening fruits, departures from which should be noteworthy.

The ready identification of the form of these records with the conventional senescent phases of respiration makes it clear that the records are devoid of initial phases which would indicate a change of physiological state as an immediate result of isolation or cooling. Hence the initial rates of the records may be taken as approximations to rates characteristic of the fruit before detachment from the plant. A series of such initial rates obtained for fruits isolated at various states of maturity ought, therefore, to indicate the manner in which the respiration is changing whilst the fruit is on the plant. This in turn should enable us to determine the relation between the course of respiration on the plant and the course after isolation at the storage temperature, as well as any changes which the seasonal factor may induce in this relation. The initial rates of these two fruits are of the order of 50–60 cc. CO_2 per kg.-hr.

Records 3 and 4 are of fruits which were picked just as the ripening color changes began, and at picking were classified as in Stage IV of maturity, very slightly in advance of Fruits 1 and 2. The respiration records are correspondingly similar and clearly represent the conventional senescent respiratory phases accompanying, in the same manner as before, the visible changes of color. The initial rates are also of the same order as those of Records 1 and 2.

The next pair of records (5 and 6) are of fruits picked in the yellow-orange or fifth stage of maturity. These records are quite different from the four that have just been examined. The initial rates are of the order of 90 cc. CO_2, which is approximately that of the peak values of Records 1 to 4, and the form of the records is that of a simple, gentle decline. In Fruit 6 breakdown occurred two weeks later than in Fruit 5. The change in color from yellow-orange, to orange, to red, accompanies these falling rates just as it accompanies the declining respiration phase in Records 1 to 4.

Judging from the initial rates of Records 5 and 6, respiration on the plant has increased substantially between Stages IV and V. We conclude, therefore, that Fruits 5 and 6 had already passed into the senescent rise of respiration before they were picked, that they were picked near to the peak of the rise and that their records of respiration after picking represent the declining arms of senescent phases which remain to be completed, on a modified time scale, at the low temperature.

Stage VI fruit is represented by Numbers 7 and 8. The form of these records is again a decline, but this time the decline is steep. The initial rates of about 100 cc. CO_2 per hr. are very high, indicating probably a further increase over Fruits 5 and 6 of respiration while still on the plant. The conclusion suggested is that Fruits 7 and 8 were picked at or just after the maximum of the senescent rise on the plant and they then completed what remained of the decline after isolation at $12.5°$ C.

Records 9, 10 and 11 are of fruits which were full red at picking. If the indications of the other records have been properly interpreted, the larger part of the senescent rise and fall of respiration in these fruits should have been passed through on the plant and we should, therefore, expect that the terminal portions only would remain to be gone through in isolation. The form and pitch of Records 9, 10 and 11 obviously fulfil this expectation.

Seven growing-green fruits of Population II were examined, and their respiration records are numbers 12 to 18. The first three were picked at the end of the growing-green stage; the remaining individuals were much less advanced in that stage when they were removed from the plant.

Records 12 to 14 begin with a more or less steady or slightly declining phase, introduced in Record 14 by an initial rise. The steady phase continues with more or less random fluctuation for 20 to 25 days, after which the rate of respiration goes up. This increase in rate is correlated in each record with the external ripening color changes in the fruit, hence we must conclude that the rise and subsequent decline represent the senescent rise of respiration. These records differ in form from Records 1 to 4 (mature-green fruit) essentially in the presence of the extended steady phase antecedent to the senescent rise. The presence of the steady phase suggests that the fruit passes through a similar phase while on the plant. If so, then Fruits 12 to 14 must have been picked well before this phase was past, so that they continued in it for 20 days after isolation before passing into the senescent phase. Fruits 1 and 2, on the other hand, must have been picked after this phase was over, for they are in the senescent rise from the beginning of the record. Moreover, the initial rates of Fruits 1 and 2 are of the same order as those of 12, 13 and 14, *i.e.*, 50 to 60 cc. CO_2, so that whatever interval of development on the plant separates the two groups of fruits, it must be an interval of relatively steady respiration.

If Fruits 12, 13 and 14 were picked just as they reached the border line between late growing- and mature-green, Fruits 15 and 16 were certainly remote from the border line and were in fact the youngest fruits to have been examined in any of the populations. Fruits 17 and 18, though assigned with Numbers 15 and 16 to the physiological Class I (early growing-green) were considerably farther advanced than the latter. They were taken from the field to supplement the fruits of Population II because at the time of the experiment, the greenhouse crop did not afford examples of fruit at precisely the right stage.
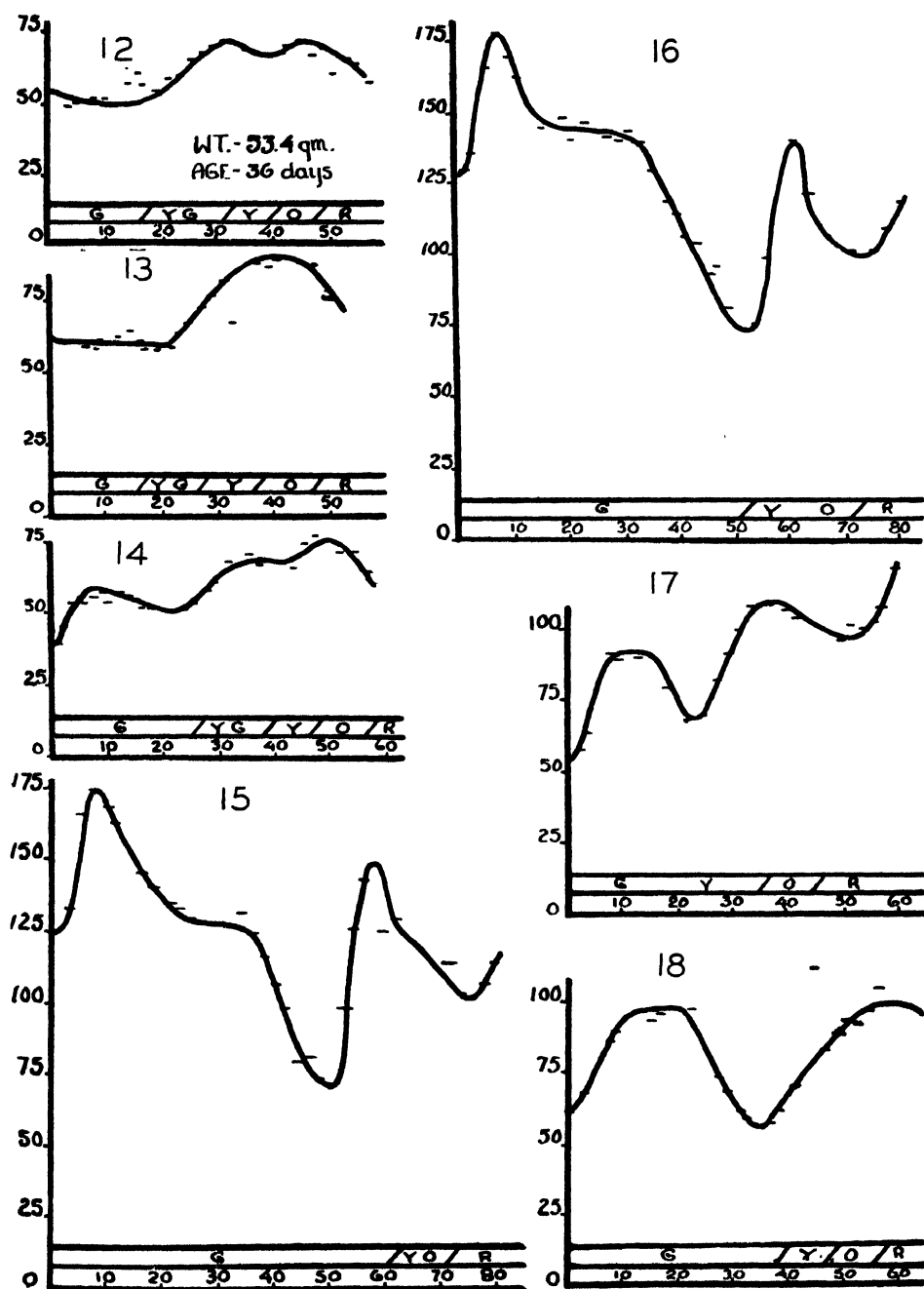
FIGS. 12–18. *The respiratory drift in storage (at 12.5° C.) of fruits of Population II. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

Dealing first with the physiologically younger pair of fruits, Numbers 15 and 16, inspection of the respiration record reveals an extremely complex form, yet one which is evidently characteristic of the state at the time of isolation, since the two records correspond in every detail. The records start off with initial values of about 130 cc. CO_2. and for the first week respiration rises until a maximum of about 180 cc. CO_2 is reached. Then a fall sets in, which is interrupted by a temporary steady state lasting for about 10 days, and then resumed until a transitional minimum of about 70 cc. is reached after 50 days. The minimum gives way at once to a sharp rise which is correlated in time with the first visible ripening color changes and is, therefore, to be identified as the senescent rise. This soon reaches a maximum and the records terminate in the manner of Records 1 to 4.

For our present purpose it is not necessary to enter more fully into the characters of these records and their physiological significance. They seem to be special cases of the general form which also underlies the respiration records of starving leaves and immature apples. In conformity with this, the initial rates of 130 cc. CO_2 are very high, indicating an altogether higher order of respiratory rate in the young growing fruit on the plant than at any subsequent stage of development.

Records 17 and 18 begin with an initial rise similar to that found in Records 15 and 16 and also in Record 14. Since this cannot be the senescent rise, it must have some other significance. Experience shows that this initial effect is characteristic of fruits isolated in the early stages of development when starch is abundant. As starch diminishes, this feature disappears from the records. Amongst the present data it is found in the records of all the very starchy fruits, in one out of three of the fruits which are rapidly losing their starch, and in no others. When the initial effect is over, respiration is found to be in a steady state similar to that which interrupts the declining phase in Records 15 and 16. After the steady phase the second limb of the declining phase appears, followed by a transitional minimum and the senescent rise. The form of Records 17 and 18 is therefore that of Records 15 and 16 with the first half of the declining phase omitted. The initial rates are of a very much lower order, suggesting that the interval separating the stage represented by Fruits 15 and 16 from that represented by Fruits 17 and 18 is characterized by a very rapid fall of respiration in the fruit on the plant.

We may now consider the whole series of summer-grown fruits. Fig. 36 is a somewhat schematized plotting of the initial rates of the isolated fruits against time. This graph represents approximately the sequence of changes in respiration rate which characterizes the growth and development of the summer-grown fruit on the plant. When we compare the series of respiration records given by isolated fruits with this graph and with each other, it is clear that the isolated fruit continues in modified form at the storage temperature the sequence begun on the plant, and does not recapitulate phases that have already been passed through. The records of fruits picked in later stages of maturity may therefore be regarded as derived from those of fruits

picked in earlier stages, by progressive omission of the early phases of the record. The metabolic history at the storage temperature is therefore determined by the point reached in the normal sequence at the time of picking.

The normal sequence of the tomato fruit growing and maturing on the plant is of the same form as that made familiar by extensive investigations on the apple and pear, and the physiological changes underlying it are presumably of general significance. The rule that the isolated fruit continues the sequence (in modified form) seems also to apply to other fruits, so that the summer-grown tomato gives no evidence of unconventionality in these respects. It is, therefore, interesting to find that autumn-winter-grown tomatoes, isolated in certain stages of development, appear to depart from this rule in a manner and to an extent that seems to have significance certainly for the scientific and possibly also for the practical aspects of the problem of preserving the fruit in the living state.

Fruits of Population III

The anomalous behavior of fruit from this as well as from subsequent autumn-winter populations of plants draws attention to the seasonal differences in growth conditions with which this variation appears to be correlated. The components of the seasonal factor for which some records are available are temperature and illumination.

The records of temperature in the greenhouse are incomplete. But fortunately it is not difficult to chart the course of mean temperature during the missing portions of the records from experience with sufficient certainty for the present purposes. In Fig. 37 the weekly mean maxima and minima are given separately for day and night. With the temperature chart is another which shows the whole growth period of each population and the interval of time during which the fruits were sampled. A similar set of charts for weekly mean hours of light and weekly mean hours of sunlight in relation to growth and sampling is given in Fig. 38. From these figures it is possible to form some conception of the manner in which these components of the seasonal factor varied from one population to another.

It is especially clear in connection with illumination that the autumn-winter population was grown under conditions that were radically different from those obtaining during the growth and sampling of the others. The summer populations were grown when this factor was rising from the minimum, and the fruit was sampled when it was maximal. The winter-spring population was grown when illumination was at first minimal, then rising, but the fruit was sampled when illumination was about half maximal. Population III on the other hand, was grown when illumination was falling from the maximum and the fruit was sampled at the minimum of this factor. There can be little doubt that in respect of light periodicity, intensity and quality, the autumn-winter period differs sharply from the summer periods, while the spring period is intermediate, but appears to have more affinity with the summer than with the autumn.

Mean temperatures tend to be steady and moderate from October to April, but reach high values in the other months of the year. In respect of temperature the autumn and winter-spring periods differ chiefly in the higher temperatures prevailing during the early growth of the autumn plants. The larger part of the two growth periods and both sampling periods were characterized by steady moderate temperature. The larger part of the growth period of the summer populations and their sampling periods were characterized by high maximum temperatures.

As a whole, the temperature and illumination components of the seasonal factor indicate a sharp difference between the summer and the autumn-winter periods, with the winter-spring period partaking of the characters of both. It might be supposed, therefore, that any influence season might have upon the physiological state of the fruit would be chiefly manifested in differences between fruits of summer and autumn-winter populations, with winter-spring fruits in an intermediate position. Owing to the fact that fruits of Stages III and IV are not represented in the winter-spring sample of the first year's work, it cannot be decided from the present body of data alone whether fruits of the autumn-winter type are found amongst the winter-spring population. But subsequent experiments show that the winter type does extend into the early spring. The transition from the winter to the summer type appears to occur in March to April.

We have little doubt that the physiological composition of the fruits of the several populations is a correlative of season. But the effect of the seasonal factor may be to influence the distribution of types which are actually present in all seasons rather than to evoke in one season a type quite unknown in another. Consequently to avoid confusing this issue until more is known we shall refer to the summer and winter types merely as the "conventional" and "anomalous" types respectively.

TABLE III

THE RELATION BETWEEN AGE AND PHYSIOLOGICAL STATE IN POPULATION III

Fruit No.	20	21	22	23	24	25	26	27	28
Stage	III	III	IV	IV	VI	VI	VI	VII	VII
Age	47	54	62	53	52	52	54	52	54
Growth interval	Oct. 3 Nov 19	Sept. 26 Nov. 19	Sept. 28 Nov. 19	Oct. 9 Dec. 1	Sept. 28 Nov. 19	Oct. 10 Dec. 1	Sept. 26 Nov 19	Sept. 28 Nov. 19	Sept. 26 Nov. 19

Table III gives for each fruit of Population III the stage of maturity at which it was picked, its chronological age and growth interval. The growth intervals vary only from 47 to 62 days and all but two lie between 52 and 54 days, yet the maturity stages represented lie between mature-green (III) and full red (VII). This sort of physiological heterogeneity amongst fruits of the same chronological age is found in other populations also but not to

the same extent as in this population.   It will be shown later that the hetero-
geneity within a single fruit is also more marked in the fruit of this than in
fruit of the other populations.

The respiration records are given in Figs. 19–28.   The first of these,
Number 19, is of a fruit which was classified at picking as on the border of
growing-green and mature-green.   A premature tissue breakdown associated
with rising respiration developed in this fruit.   Nothing of the sort occurred
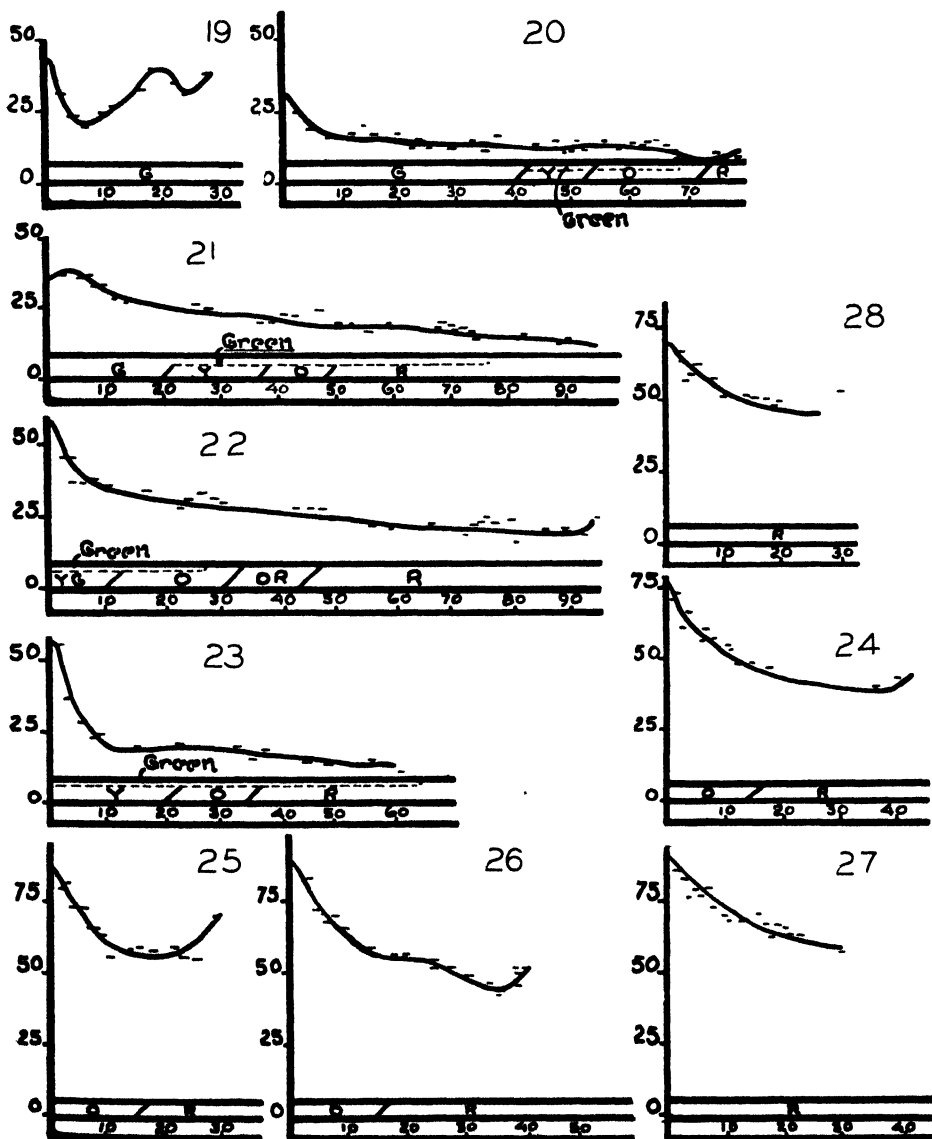
FIGS. 19–28.   *The respiratory drift in storage (at 12.5° C.) of fruits of Population III.
Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes
in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

in any other fruit of this population. The breakdown did not appear to have an infective origin, but there is no critical evidence that this is so. It might be characteristic of an extreme variant in a physiologically heterogeneous group. It is best, therefore, to reserve comment on this individual.

Fruits 20 and 21 were picked in the mature-green stage. The respiration records are low in pitch and the initial rates are of the order of 35 to 40 cc. CO_2. Record 21 gives slight indication of an initial rise such as characterized the earlier stages of corresponding fruits in Population II. From the initial value the rate drops more or less gently to a prolonged phase of low, almost steady, slightly declining respiration. This phase is of remarkable duration. The experiments had to be terminated before it came to an end and before the fruits showed the slightest sign of breakdown. The records, though very long, are nevertheless incomplete. In fruit of later populations records of this type have been observed which went on for 130 days.

The only other fruits to pass through a long steady phase of anything like this appearance were Numbers 12, 13 and 14 of Population II. But in the latter, the steady phase passed into the senescent rise when the external color changes began, whereas in the present fruits the steady phase continues quite unbroken throughout the period of color change and long past the point at which the fruits had become full, ripe red. The only unusual feature of the ripening color changes was a marked tendency of an island of tissue in the immediate vicinity of the calyx to lag behind the rest of the fruit. Ultimately ripening was complete. The persistence of the calyx end indicates, however, that these fruits are characterized by a higher order of cell heterogeneity than summer-grown fruits.

It is evident that the steady phases of Records 12, 13 and 14 cannot be homologized with those of Records 20 and 21. Indeed the present records have little in common with those of summer-grown fruit picked at any stage whatever. They represent the anomalous type, the characteristics of which are a decline sometimes prefaced by an "initial effect" passing into a phase of steady respiration greatly extended in time, during the unbroken course of which the ripening color changes occur. The essence of the anomaly is the absence of the senescent rise, a phenomenon occurring regularly in relation to ripening not only in our summer-grown fruit but also observed as the rule for tomatoes by Gustafson (4). This type of record appears to indicate a state of physiological stability quite unusual for tomatoes and is associated with a duration of life at 12.5° C. several times as great as that characterizing corresponding fruits of the conventional type.

The next two fruits (Records 22 and 23) were isolated at Stage IV, just as they were commencing to turn yellow. The records are of the same general form as Numbers 20 and 21. The initial rates of 57 and 60 cc. CO_2 are higher than those of Records 20 and 21, from which it appears that while yet on the plant the fruits actually pass into the usual rising phase of respiration as the ripening color changes supervene. In this they resemble the conventional type. But the remarkable thing is that the yellowing fruits of the present

population, after they are picked, do not proceed with the senescent rise in the manner of the conventional type (Records 1 to 4). On the contrary, the records decline from the beginning, passing into an extended phase of precisely the character of that in Records 20 and 21. The ripening color changes also occur in the absence of a senescent rise, and the extraordinary duration of life which accompanied the stable state of Numbers 20 and 21 occurs in the present pair as well.

This series of records does not happen to include examples of fruits isolated in Stage V. Records 24 to 28 are of fruits picked in the orange (VI) and red (VII) stages of maturity. The first three were orange when picked. In the fruit of the summer populations this color, whether developed on the plant or after picking, at 12.5° C. is associated with the peak values of the senescent rise of respiration. This appears to be true of the attached autumn-winter
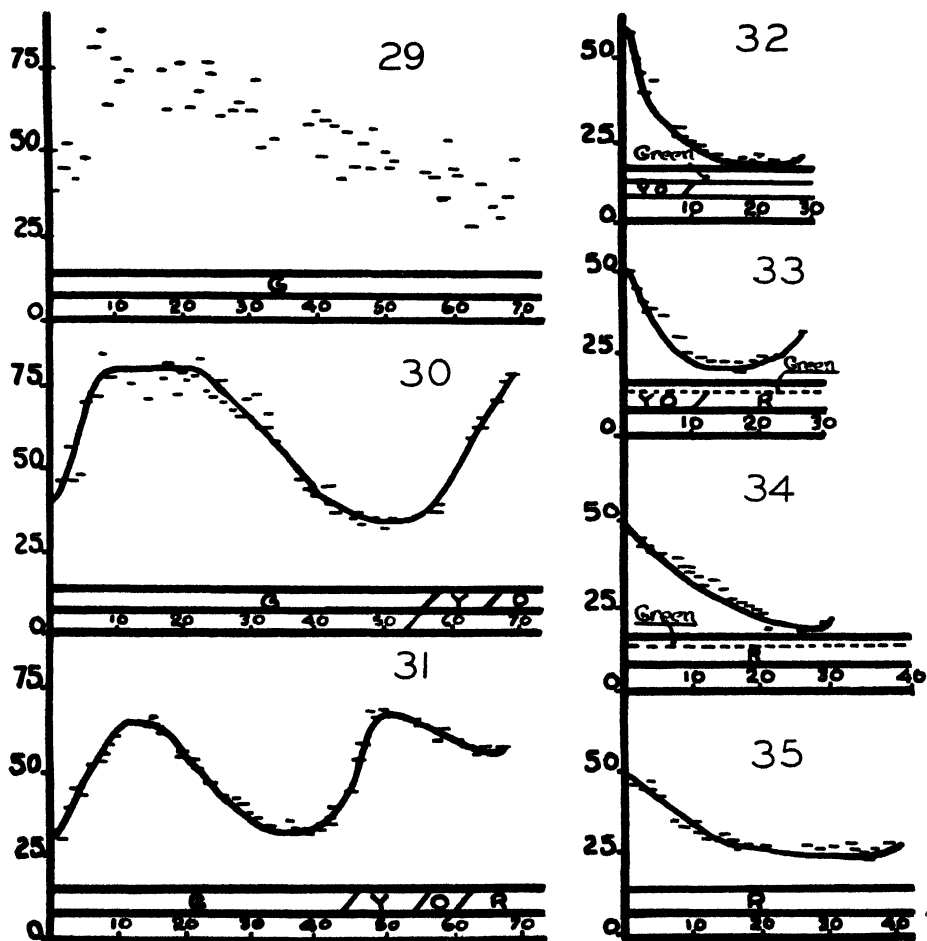
FIGS. 29–35. *The respiratory drift in storage (at 12.5° C.) of Population IV. Vertical axis indicates cc. CO_2 per 10 kg. per hr.; horizontal axis, time in days and color changes in the fruit (G = green, Y = yellowing, YO = yellow-orange, O = orange, R = red).*

fruits also for we find the initial rates of Records 24 to 26 in the vicinity of 80 to 90 cc. CO_2. The form of the records is that of fruits of corresponding maturity in the summer populations and, as before, evidently represents the declining arm of the senescent phases. No stable state is established and in 25 to 40 days breakdown occurs.

Of the two fruits picked full red, Number 27 has a high initial rate and a record very much the same in form as those of fruits that were orange when picked. Presumably it was actually on the border line of orange and red. The record of the other ripe fruit has a lower initial rate and in all its characteristics is indistinguishable from the corresponding ripe fruits of Population I. (Records 9 and 10). It would seem, therefore, that the stable characteristics which distinguish the isolated unripe fruits of autumn-winter populations from the corresponding summer-grown fruits are not manifested in the autumn-winter fruit if it is permitted to pass a certain point of maturity before it is picked. The evidence is that this point is roughly marked by the external appearance of red pigmentation in the fruit.

Fruits of Population IV

This is the winter-spring population and the records of respiration of the fruits studied are given in Figs. 29–35. The first of these is characterized by an unusual scatter of points referable to error in the measurement of the low
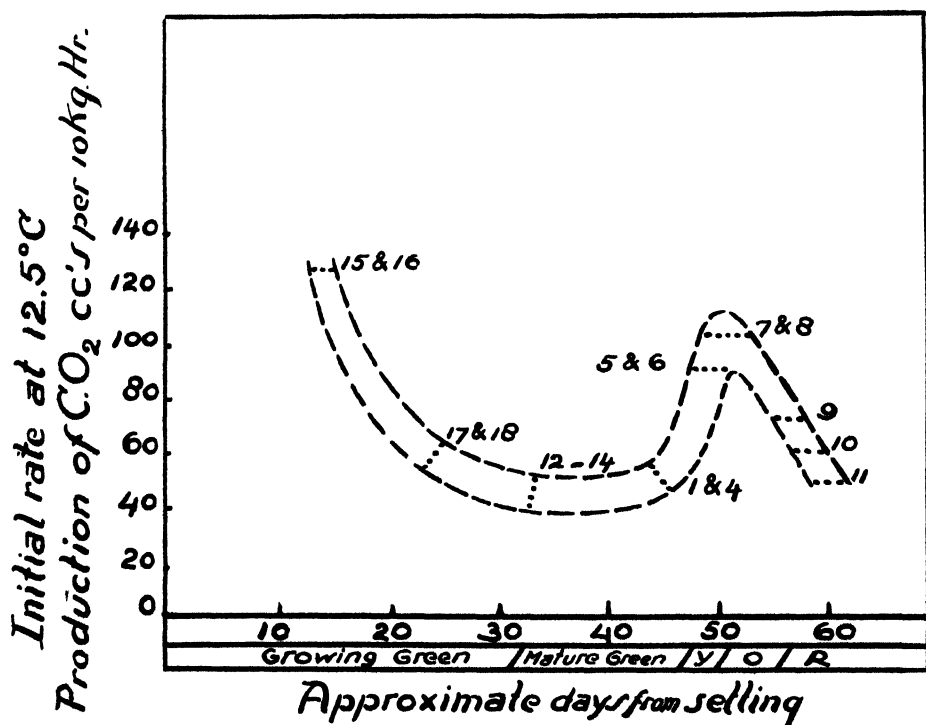
FIG. 36. *The drift of respiration of tomato fruits of Populations I and II on the plant as determined from the initial rates of the stored fruits of the summer-grown populations.*

CO_2 output of the very small fruit. There is evidently an "initial effect" such as appears in the records of all very young fruits, and thereafter a decline, but all details of form are obscured. The two fellow records (30 and 31) are of fruits not so early in the growing-green stage and their form is evidently that of Records 17 and 18, Population II, but they are of lower pitch. We conclude that winter-spring fruit isolated in March at the growing-green stage is physiologically comparable to corresponding summer fruit.

The next stages of maturity were not represented in fruits of this population, but the examination of subsequent winter-spring fruits indicates that the anomalous type appears, provided the fruits are picked early in the spring as well as at the appropriate stage of maturity. Later, the conventional type predominates.

Fruits 32 and 33 were picked just as the red pigment began to appear superficially. Their relatively high initial rates indicate that the senescent rise had set in while they were still on the plant. The records conform to the conventional type and are comparable to Records 5 and 6 of Population I except again for their lower pitch. Similarly Records 34 and 35 of fruits picked full red are comparable to Records 9, 10 and 11 of Population I.
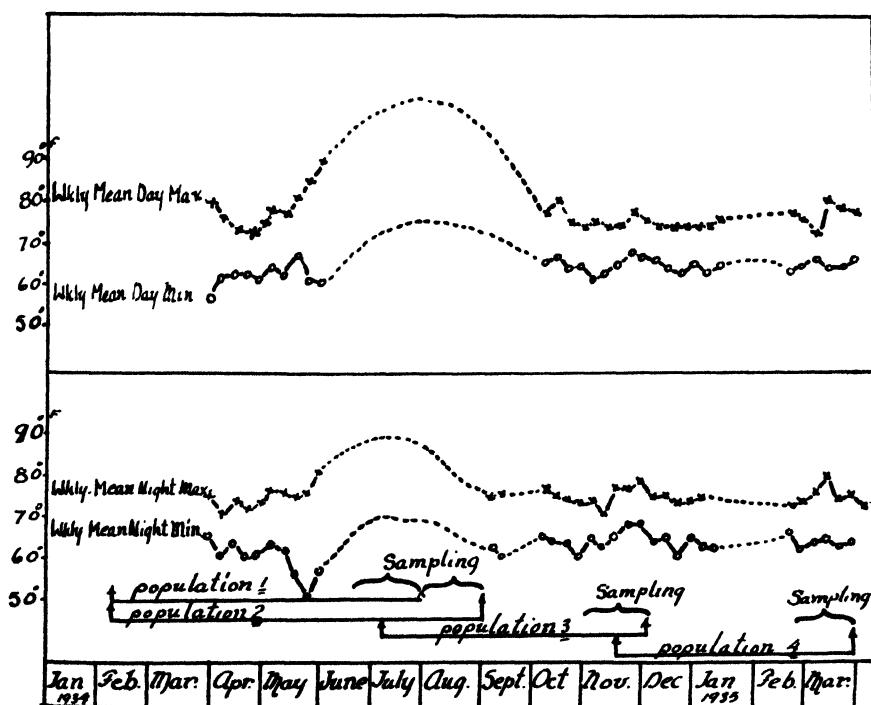
Fig. 37. *Temperature records in the greenhouse during the growth of Populations I to IV, showing the weekly mean day maximum, the weekly mean day minimum, the weekly mean night maximum and the weekly mean night minimum. These temperature records are not complete but are connected by dotted lines which indicate the probable temperatures approximating those obtained from outside temperatures. The period of growth and the sampling period for each population are indicated at the bottom of the figure.*
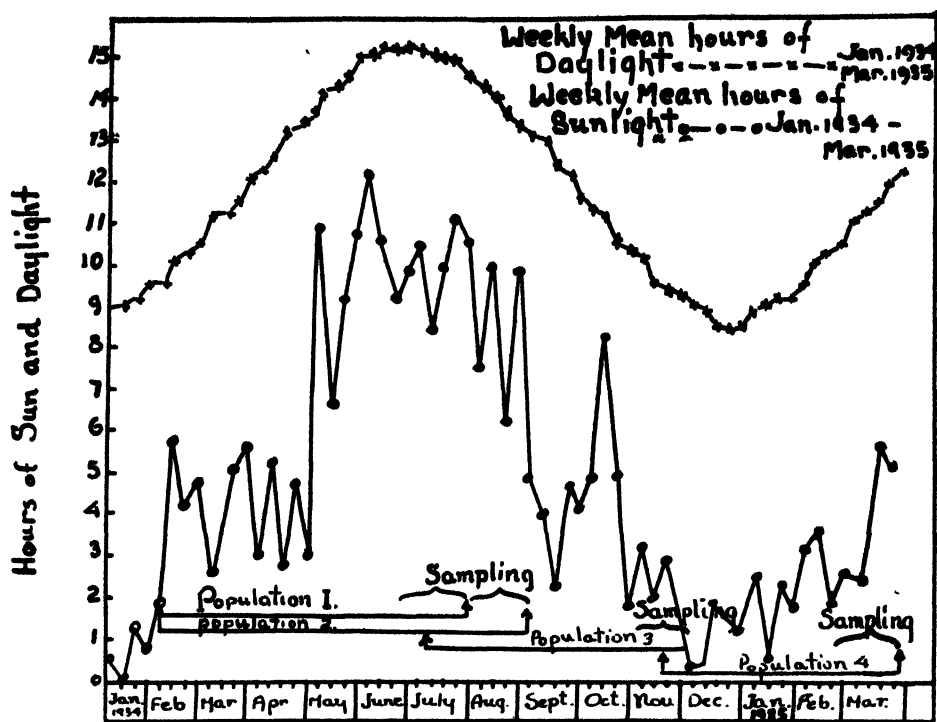
FIG. 38.   *The weekly mean hours of daylight and the weekly mean hours of sunlight, together with the growing period and the sampling period for each of the four populations.*

The evidence provided by the data examined in detail in this paper, as well as of those for subsequent populations of tomatoes, indicates that in the course of ripening while on the plant, fruits pass through the senescent rise of respiration irrespective of the time of year in which they are grown in the greenhouse.   During the late spring and summer the ripening processes of isolated fruit at 12.5° C. are also accompanied by the senescent rise of respiration in the conventional manner and are characterized by the lack of durability normal to this fruit.   At other times of the year the course of metabolism in the fruit isolated at 12.5° C. depends upon the stage of maturity at which it is picked.   If ripeness is approaching, as indicated by the external appearance of red pigment, the course of metabolism conforms to the conventional type and the fruit is not durable.   But the autumn-winter-early-spring fruit, if picked approximately mature-green, departs from the conventional type.   The respiration record reveals an extended period of remarkably steady rate while ripening is in progress and for long afterwards, suggesting a metabolically stable condition quite different from that found in fruit of the conventional type.   This anomalous type of ripening is associated with a very much greater durability of the fruit both at 12.5° C. and afterwards, if removed to a higher temperature.   The quality of such fruit in respect of texture and flavor when ripe is quite satisfactory.

Acknowledgments

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PHYSIOLOGY OF APPLES IN ARTIFICIAL ATMOSPHERES[1]

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INTRODUCTION

The influence of artificial atmospheres upon stored fruits has been the subject of much investigation during the past decade, not only in relation to the practical problems but also to those of a more fundamental character concerned with the living activities of the plant. The notable work of Kidd and West (20) has seen its fruition in the present commercial development of gas storage for apples, and their studies together with those of Blackman and others (3, 14, 32, 37) have greatly contributed to the knowledge of the process of plant respiration.

This report includes the following considerations:

1. Respiration.
2. Biochemical changes.
3. Osmotic and permeability relationships.
4. Fungal activity.
5. Physiological disorders.

The results of the above investigations will be dealt with under separate headings.

MATERIALS AND METHODS

Storage Methods

Three varieties of apples were used throughout the main experiments; namely, McIntosh, Cox Grange and Golden Russet. The fruit was first placed in storage at 0° C. immediately after picking, and then removed to cellar storage conditions at a mean temperature of 4.5° C. at the end of October 1935. At this time samples of fifty apples for each treatment were placed in five-gallon cans equipped with two copper tubes, one of which extended to the bottom of the container; these were used for sampling purposes. The lids of the cans were heavily vaselined and thus rendered gas tight. Carbon dioxide and nitrogen were added directly from cylinders under pressure, the latter being used to reduce the normal concentration of oxygen. An Orsat gas analysis apparatus accurate within 0.2% was used for the analysis of the atmospheres. Analyses of the atmospheres were made daily and the containers ventilated in order to maintain the desired concentration of carbon dioxide. At the end of December 1935, the material was moved from the Kentville Experimental Station, N.S., to a storage cellar at Macdonald College, Quebec, where the temperature averaged 3° C. (±1°).

The treatments and controls in air which were used are shown in the adjoining table.

Variety	Per cent CO_2	Per cent O_2	Per cent N_2	Average gas concentration during entire storage period	
McIntosh	2.5	18.5	79	2.6 CO_2	
McIntosh	5.0	16.0	79	4.5 CO_2	
McIntosh	10.0	11.0	79	9.1 CO_2	
					Duplicate
Cox Orange	—	2.5	97.5	2.1 O_2	2.5 O_2
Cox Orange	2.5	18.5	79	2.4 CO_2	3.1 CO_2
Cox Orange	5.0	16.0	79	4.8 CO_2	5 2 CO_2
Cox Orange	10.0	11.0	79	9.5 CO_2	9 6 CO_2
Golden Russet	5 0	16.0	79	4.0 CO_2	
Golden Russet	10.0	11.0	79	9.2 CO_2	

Two examinations were made, 25 apples being used in each case. In the first examination 10 fruits were frozen at $-15°$ F. and the tissue ground up and stored in bottles for one week in the frozen condition until used for subsequent experiments. Another 10 fruits were set aside for acid, hydrogen ion concentration, and refractometric determinations.

In addition, several subsidiary experiments were undertaken in which the concentrations of carbon dioxide and nitrogen were adjusted to high levels for limited periods.

Respiration

The evolution of carbon dioxide from the fruit was measured by means of the weighed tube method, the equipment and methods being similar to that described by the author in a previous paper (10) with some modifications. The carbon dioxide absorbent, "Ascarite", a Central Scientific Company product, was substituted for the soda flake. This absorbent is the American equivalent of the B.D.H. product, "Carbosorb", both of which are used extensively in organic analysis. Figure 1 indicates the
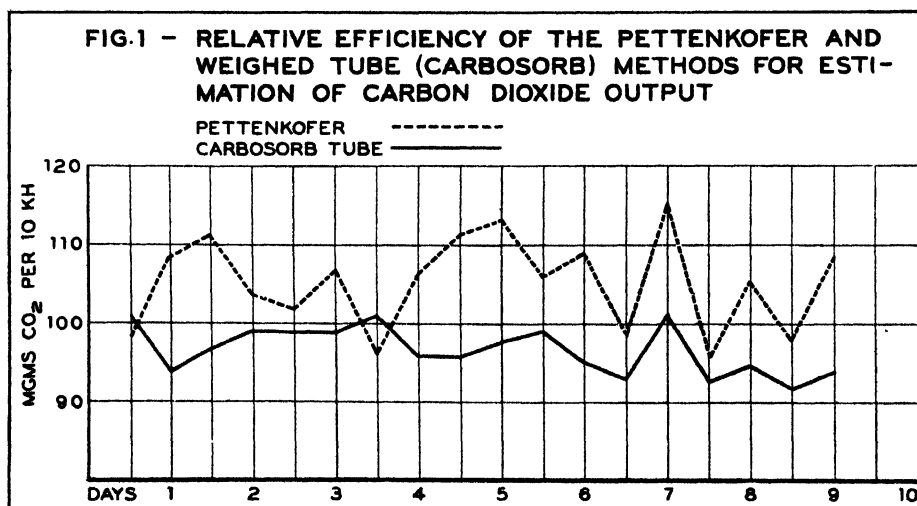
FIGURE 1. Relative carbon dioxide absorption by the weighed tube and Pettenkoffer methods for respiration measurements at 10° C.

relative efficiency of "Carbosorb" as compared with the Standard Pettenkofer method in which the carbon dioxide is absorbed by barium hydroxide. A small "Marco" pump was used to drive the air through the system.

The incoming air was passed through a large tube of soda lime and conditioned to a relative humidity of 75% by bubbling through a solution of 30% potassium hydroxide. After passing over the fruit the air was dried by means of two tubes of coarse- and fine-mesh calcium chloride respectively. A flask containing a weak solution of sodium hydroxide plus methyl orange was placed at the end of the train in order to detect improper absorption and to check the speed of the air flow (2 litres per hour). The entire apparatus is shown in Figure 2.

FIGURE 2.   Apparatus for the estimation of carbon dioxide output of apples.

Estimations of the carbon dioxide output and oxygen uptake of fruit in the five-gallon containers were made by means of the Orsat apparatus.

RESULTS

Respiration

The measurement of the respiratory activity of fruit stored at low temperatures by means of gas analysis was considered to be unsatisfactory owing to the very slow rate of carbon dioxide output obtained under such conditions (see Figure 6). The first experiment, using the gas analysis method, was therefore carried out at 21° C.

The object of this experiment was to ascertain the effect of artificial atmospheres upon apples belonging to the Stark variety which had been stored in air at 3° C. for approximately four months. Samples of fruit, each weighing 2240 grams, were removed from storage and placed in containers the lids of which were sealed very thoroughly with a layer of vaseline 0.25 inch in thickness.

This method of obtaining a record of carbon dioxide is relatively crude when compared with the Pettenkofer or weighed tube method. Never-

theless it was felt that the use of such large samples, approximately 30 apples, would serve to minimize the error obtained by using single fruits. Care was taken to avoid upsetting the internal atmospheres, particularly the high carbon dioxide container, in which a negative pressure was developed at the time of analysis. In view of these reservations attention was given only to total carbon dioxide output and oxygen consumption over the period stated.

Four treatments were used and the changes in the atmospheres noted after 5 days. The containers were then thoroughly aerated and closed again for a further 4 days. Table 1 indicates the atmospheric changes found under these conditions.

TABLE 1.—CARBON DIOXIDE OUTPUT AND OXYGEN UPTAKE OF STARK APPLES AT 21° C. IN DIFFERENT ATMOSPHERES (PER CENT DECREASE AND INCREASE IN CONTAINERS)

Treatment	In artificial atmosphere (5 days)		In air (4 days)		Respiratory quotient in air	
	CO_2 increase	O_2 decrease	CO_2 increase	O_2 decrease	Initial	Final
(1) 6.2% CO_2	14.3	11.0	10.7	7.9	1.66	1.08
(2) 53.5% CO_2	9.0	7.0	17.5	10.7	2.14	1.00
(3) 99.0% N_2	15.3	1.0	11.3	6.6	1.66	1.15
(4) Control in air	11.7	8.2	9.4	7.0	1 60	1.20

It will be seen that the total carbon dioxide output from fruits placed in high concentrations of carbon dioxide is less than that shown by the controls, but that upon removal to air the relationship is reversed as seen by the initial respiratory quotient in air of 2.14. This is followed by a sharp readjustment to an R. Q. of unity which would indicate an initial expulsion of carbon dioxide from the tissues.

On the other hand low carbon dioxide (6.2%) and nitrogen (99.0%) treatments are characterized by a greater increase in carbon dioxide output than the control which is also maintained upon removal to air. The oxygen consumption of these lots when removed to air differs in that the uptake of nitrogen-treated apples is lower than either the low carbon dioxide treatment or the controls. It may be further noted that the oxygen consumption of the high carbon dioxide treatment when removed to air is much in excess of the other lots.

Samples of McIntosh apples were also carried under similar conditions' but trouble was experienced with pressure differences when the containers were removed from cold storage to ordinary room temperatures. Nevertheless, both the Stark and McIntosh apples placed in nitrogen were characterized by a disappearance of the red pigmentation. This development, which to the writer's knowledge has not been reported before, is shown in Figure 3. The effect rather resembles soft scald when photographed, but is quite different in that the decolorized areas are almost white in appearance as distinguished from the sharply delimited light brown patches which are seen on scalded apples.
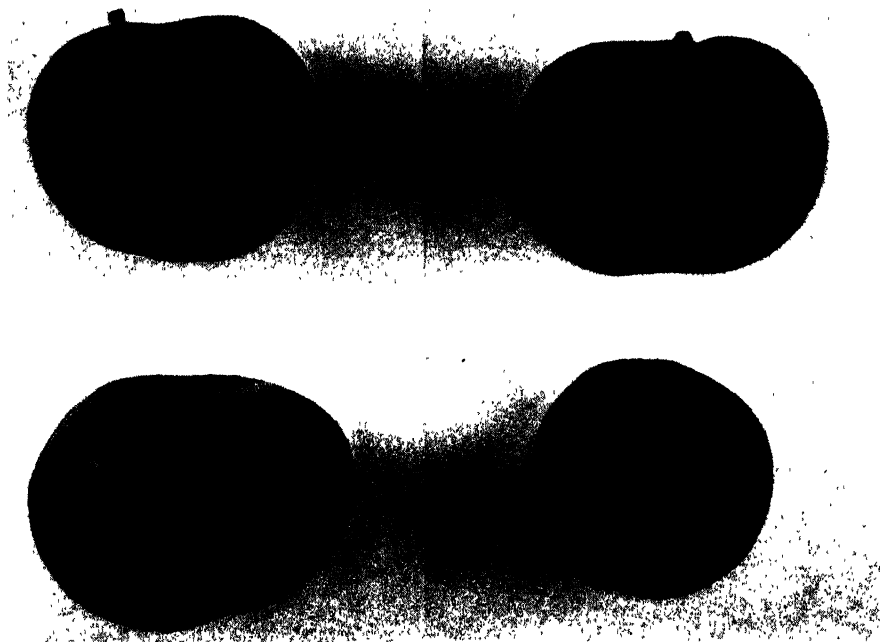
FIGURE 3. Loss of red pigmentation in senescent McIntosh apples stored in 100% nitrogen at 21° C.

The relative respiration rates of Golden Russet apples stored at 3° C. and 21° C., as determined by the weighed tube method, are shown in Figure 4, but the differences due to treatment with carbon dioxide are not
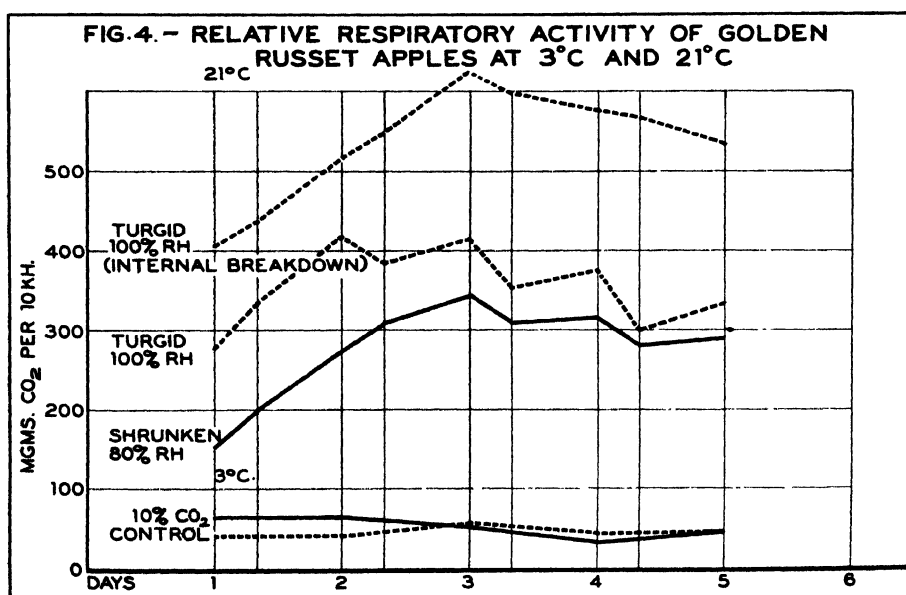
FIGURE 4.   Relative respiratory activity of Golden Russet apples at 3° C. and 21° C.

clearly defined. It will be seen, however, that the respiratory ratio of fruits at 3° C. and 21° C. is in the order of 1 : 6 respectively. This ratio closely approximates the 1 : 5.35 ratio obtained by the writer (10) with Bramley's Seedling apples removed from 3° C. to 18° C. Furthermore, fruit stored in air with a relative humidity of 100% results in a higher rate of respiration over that shown by the controls in air at 80 to 85% R. H. when removed from 3° C. to 21° C. The great increase in carbon dioxide output of fruits affected by internal breakdown is also worthy of note.

In order to evaluate the size effect, not only in regard to carbon dioxide output but also to moisture loss, the three treatments, namely, shrunken controls, turgid controls, and those apples stored in 10% carbon dioxide, were duplicated using large and small fruits (three apples per sample). For determination of moisture loss the fruit was weighed both at the commencement and at the end of the experiment.

TABLE 2.—TOTAL CARBON DIOXIDE AND MOISTURE LOSS OF GOLDEN RUSSET APPLES AT 21° C.

Treatment	Weight of sample (in grams)	CO_2 loss (mgms. per kilo.)	Moisture loss (mgms. per kilo.)	Moisture/ CO_2
Shrunken (85% RH)	266.65	2503	8362	3 3
Shrunken (85% RH)	150.34	2649	9376	3.5
Turgid (100% RH)	256.84	3238	6262	1.9
Turgid (100% RH)	180.87	4600*	16497*	3 5
Turgid (10% CO_2)	210.23	3299	8525	2.6
Turgid (10% CO_2)	158.68	2912	9692	3.3
			Average	3.0

* Apples affected with internal breakdown.

Table 2 indicates that size is an important factor in the determination of relative rates of transpiration in that the ratio of surface area to bulk decreases with size increase, and thus the smaller fruits exhibit higher rates of transpiration than the larger fruits. The size effect is not so marked in relation to respiration in this test, and may be due to diffusion interference as brought about by the senescent condition of the internal tissues of the fruit. It is of interest to note that the ratio of the rates of respiration and transpiration in Golden Russet apples is approximately 1 : 3.

The first test with Golden Russet apples failed to clearly differentiate the carbon dioxide and high humidity effects. The experiment was therefore repeated, omitting the shrunken fruits; the two series were run in triplicate again using large and small apples. It will be seen from Figure 5 that the fruits stored under high humidity conditions respired more rapidly than those removed from 10% carbon dioxide (both previously stored at 3° C.); in addition, the moisture loss of the former sample was found to be 1.7 times greater than in the latter. Size relationships were again apparent in this experiment, but the results shown in Figure 5 are on an equivalent weight basis.

FIG.5 – RELATIVE RESPIRATORY ACTIVITY OF GOLDEN
·RUSSET APPLES AT 21°C.   STORED IN AIR 100%RH
AND 10% CARBON DIOXIDE
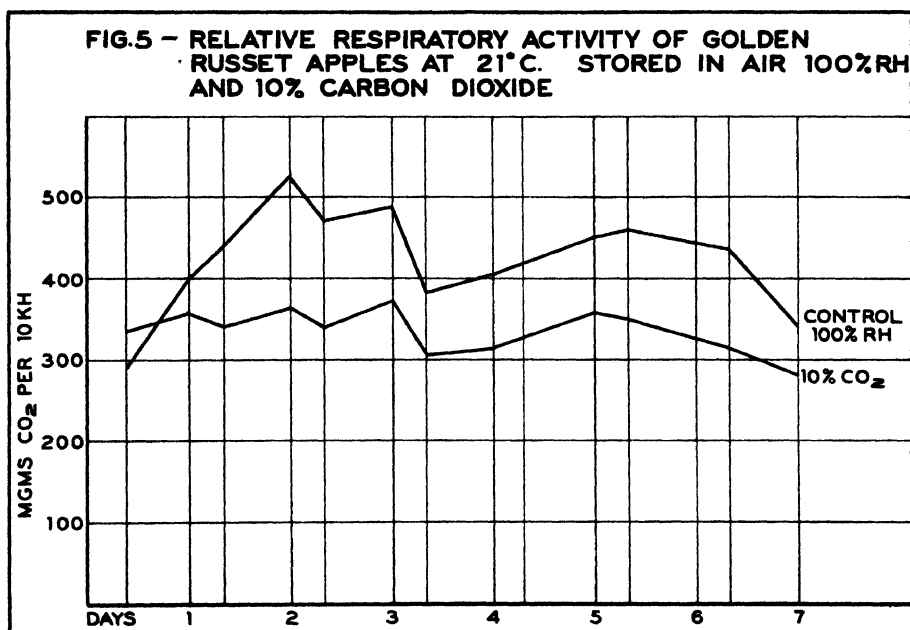
FIGURE 5.   Relative respiratory activity of Golden Russet apples at 21° C.
Stored in air 100% RH and 10% carbon dioxide.

Apples of the McIntosh variety stored in 5 and 10% carbon dioxide
and in air at 3° C. were also transferred to 21° C. for respiration observations
and were run in duplicate.   Figure 6 shows that the carbon dioxide effect

FIG. 6 – RELATIVE RESPIRATORY ACTIVITY OF MᶜINTOSH
APPLES AT 21° C.
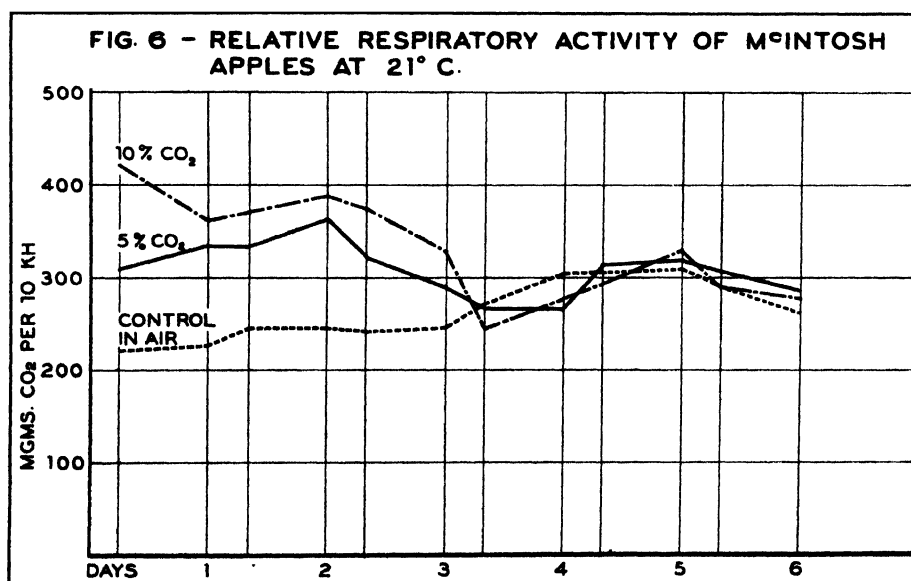
FIGURE 6.   Relative respiratory activity of McIntosh apples at 21° C.

is similar to that found with Golden Russet apples except that the McIntosh fruits appear to lose the effect of the gas more rapidly as both the treated and untreated samples respire at the same rate after three days.

Biochemical Changes

Determinations were made on the hydrogen ion concentration and the total acidity (expressed as malic acid); carbohydrates were estimated on frozen macerated tissue. Moisture content was also determined, but the data obtained has been linked up with osmotic relationships of the treated fruits.

The juice was expressed from 10 apples in each treatment by means of an ordinary food grinder and a small hand press. Hydrogen ion concentrations were determined with a quinhydrone electrode apparatus, and the total acidity by titration against a standard solution of 0.1 N sodium hydroxide using phenolpthalein as the indicator.

Sugars were determined by the modified Munson and Walker (26) method which depends on the ratio of copper reduction to sugar oxidation in an alkaline solution. The major modification of the original method is the substitution of a citrate-carbonate reagent in the place of the Fehlings solution (Shaffer and Somogyi (29), Scoggan (30)). The first pH estimations were made on the treated fruit which was held in common storage, the results being shown in Table 3.

TABLE 3.—pH VALUES OF EXPRESSED JUICE OF APPLES HELD IN ARTIFICIAL ATMOSPHERES
IN COMMON STORAGE, 3° C. TO 4.5° C.

Variety	Date examined	Control in air	O_2 2.5%	CO_2 2.5%	CO_2 5.0%	CO_2 10%
Russet	30/1/36	3.72	—	—	3 64	3.64
Russet	12/4/36	3.81	—	—	3.81	3.81
McIntosh	30/1/36	3.55	—	3.38	3.38	3.38
McIntosh	12/4/36	3.76	—	3.69	3.64	3.60
Cox Orange	30/1/36	3.64	3.81	3.59	3.64	3.55
Cox Orange	31/4/36	3.94	3.81	3.72	3.98*	3.81

* Apples affected with severe internal breakdown.

The results in Table 3 indicate quite clearly that the hydrogen ion concentration decreases as senesence proceeds, with the fall particularly marked in the McIntosh and Cox Orange apple varieties in which the activity during senescence is much more pronounced than in the Russet variety. With one exception the influence of carbon dioxide appears to be quite consistent, namely, a lower pH value than that obtained in the controls. It has been stated that the method of determination may cause a loss of carbon dioxide, but even if this were the case one might reasonably expect yet lower pH values than those obtained. There are no large differences in the values obtained between the effects of the various concentrations of carbon dioxide; those obtained in the last examination of Cox Orange apples are influenced by the development of incipient breakdown. The

FIG. 7 — INFLUENCE OF ARTIFICIAL ATMOSPHERES ON FUNGAL ACTIVITY IN STORED APPLES
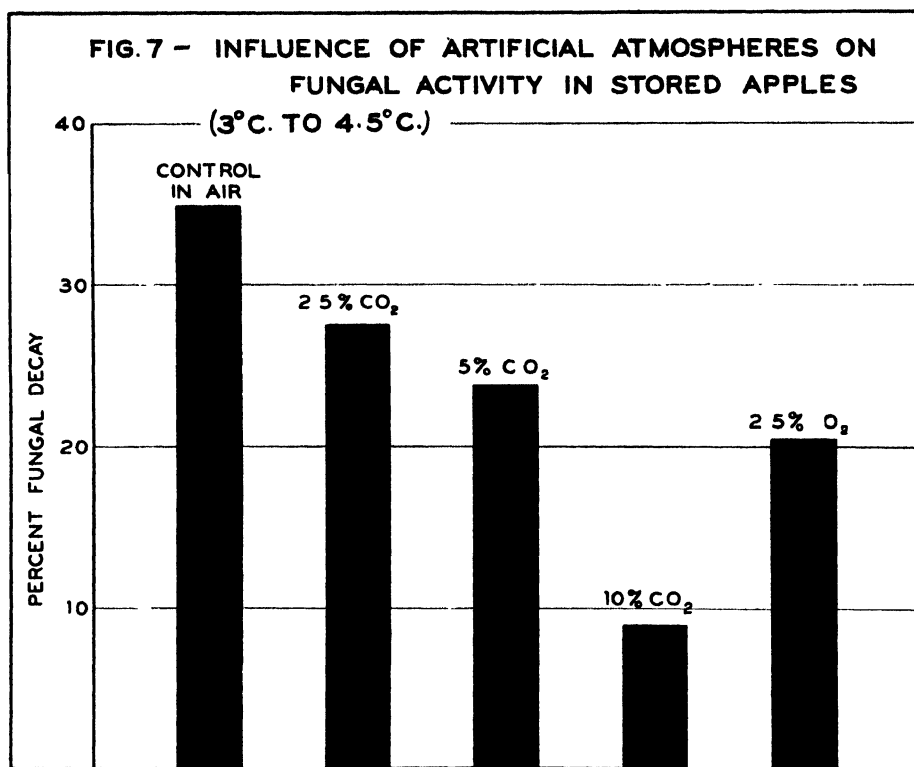
FIGURE 7. Influence of artificial atmospheres on fungal activity in stored apples (3° C. to 4 5° C.).

increase in alkalinity of the juice from the severely-broken-down fruits is particularly noteworthy.

It was decided to follow this investigation further, and at the commencement of the 1936 storage season samples of Gravenstein apples and Clapp Favorite pears were picked and immediately stored in containers with atmospheres of 100% carbon dioxide and 100% nitrogen for one week. The samples under the latter conditions were flushed with pure nitrogen every 24 hours until no carbon dioxide could be detected. Four temperatures were used: 0° C., 4.5° C., 10° C. and 18° C.

TABLE 4.—THE INFLUENCE OF ARTIFICIAL ATMOSPHERES UPON THE pH VALUES OF EXPRESSED JUICE OF APPLES AND PEARS AT DIFFERENT TEMPERATURES FOR ONE WEEK

Material	Treatment	Temperature			
		0° C.	4 5° C.	10° C.	18° C.
Apple	100% CO_2	3 14	3 17	3.24	3.28
Apple	100% N_2	3 10	3.05	3.17	3.09
Apple	Control in air	3.00	3.09	3.00	3.02
Pear	100% CO_2	4.17	4.41	4.37	4.55
Pear	100% N_2	3 90	4.10	3.90	4.09
Pear	Control in air	3.90	4.00	4.00	4.06

Table 4 shows that the carbon dioxide effects are reversed in relation to the results obtained in the previous experiment in that there is an increase in the alkalinity of the expressed juice which becomes more pronounced at higher temperatures. The greater change shown by the pears than the apples bears out the point that the former are able to absorb carbon dioxide to a greater extent than the latter fruits (13).

On the other hand, fruits in the absence of both oxygen and carbon dioxide show inconsistent effects, but the tendency appears to be in favour of a less acid reaction.

Estimations of total acidity were made in the course of the 1936–37 storage season on a series of treated fruits from common and 3° C. storage, save that the low oxygen and 2.5% carbon dioxide treatments were omitted, and a sample of Golden Russet apples stored in 100% relative humidity was added. The results are shown in Table 5.

TABLE 5.—PER CENT MALIC ACID IN APPLES STORED IN ARTIFICIAL ATMOSPHERE IN COMMON STORAGE (C.S.), 3° C. TO 4.5° C. FOR FIVE MONTHS

Variety	Temperature	Control in air 85% RH	Air 100% RH	CO_2 5%	CO_2 10%
Russet	C.S.	0.235	0.257	0.281	0.300
Russet	3° C.	0.372	0.359	0.370	0.360
McIntosh	C.S.	0.216	—	0.267	0.293
McIntosh	3° C.	0.317	—	0.317	0.359
Cox Orange	C.S.	0.270	—	0.289	0.320
Cox Orange	3° C.	0.349	—	0.331*	0.312†

Apples slightly* and severely† affected with internal breakdown.

Under the higher temperature conditions it will be noted in Table 5 that the carbon dioxide has retarded the rate of acid consumptiom, but that at 3° C. the differences are inconsistent although the acid content is higher than that found under common storage conditions. There is also a decrease in total acidity as well as actual acidity in fruits affected with breakdown.

With regard to the effect of humidity it should be pointed out that the fruits stored at the higher temperatures in relative humidities of 85 and 100% were very shrunken and firm respectively, whilst at the lower temperatures the degree of firmness differed very slightly in the corresponding samples. This variation in moisture loss may account for the conflicting values obtained, but this point requires further investigation.

Carbohydrate estimations were confined to the Cox Orange apples. It must be pointed out that the figures shown in Table 6 are purely indicative as the data are insufficient to

TABLE 6.—PERCENTAGES OF TOTAL, REDUCING AND NON-REDUCING SUGARS IN COX ORANGE APPLES STORED FOR 3 MONTHS IN ARTIFICIAL ATMOSPHERES AT 3° C. TO 4.5° C.

Treatment	Total	Reducing	Sucrose
2.5% O_2	10.40	7.73	3.22
2.5% CO_2	11.73	7.56	4 79
10.0% CO_2	10.50	7.32	3.73
Control in air	12.53	7.43	5.58

warrant definite conclusions being drawn. It may be seen, however, that the low oxygen concentration has apparently accelerated the loss of sucrose as found by Fidler (11), and a similar effect but less marked may be seen in the sucrose value for the 10% carbon dioxide treatment. The control in air is characterized by the least loss in total sugars of all treatments.

Observations were made on the colour changes occurring in the stored fruits and it was seen that all the carbon dioxide treatments and also the low oxygen retarded the yellowing of the ground colour on McIntosh and Cox Orange apples.

Osmotic and Permeability Relationships

The plasmolyticum used in the determination of osmotic values and of permeability was calcium chloride, as it has been shown that this solution is unable to enter plant cells. Furthermore, the solution will last several months according to Levitt and Scarth (23). The chief objection to the use of sucrose is that of nicro-organic development and this was observed in the course of these investigations. Osmotic values were estimated to within 0.01 M and a series of solution were made up from 0.1M to 1M calcium chloride.

The osmotic pressures in atmospheres were calculated from the freezing points given in the International Critical Tables according to the equation

$$OP = 12.06 - \Delta 0.021^2$$

in which OP = osmotic pressure and Δ = corrected depression of the freezing point. This equation was also used in connection with the cryoscopic determinations of osmotic pressures.

Neutral red 5 p.p.m. was adopted as the staining medium throughout these cellular studies; the cell walls remain unstained and the dead cells are easily distinguishable by the purplish granulated appearance of the protoplasm. A minimum of ten sections were used for the determination of incipient plasmolysis, this point being reached when over 50% of the cells showed this characteristic.

Cryoscopic determinations were made by means of a Hortvet cryoscope upon juice expressed from freshly thawed macerated apple tissue. Corrections were made for the true depression of freezing point using the tables of Harris and Gortner (15).

Estimations of moisture content were made on 20-gram samples of freshly thawed tissue utilizing two methods: (1) 100° C. for 24 hours, and (2) 50° C. in vacuo for 48 and 72 hours. The figures obtained for the 48-hour period in the second method have been omitted, as it was found that the moisture estimate after 72 hours drying did not exceed by more than 0.1% that obtained after 48 hours.

In Table 7 it will be seen that the pressure in pounds required to break the apple tissue with a penetrometer is included, and also the percentage of total solids as calculated from the refractive index of the juice. An Abbé prism refractometer was used to obtain the refractive index of the juice, and the corresponding percentage of total solids was derived from the International Critical Tables.

TABLE 7.—OSMOTIC PRESSURE, MOISTURE CONTENT, TISSUE RESISTANCE AND THE PERCENT
TOTAL SOLIDS (EXPRESSED JUICE) OF APPLES STORED IN DIFFERENT ATMOSPHERES
IN COMMON STORAGE FOR THREE MONTHS AT 3° C. TO 4.5° C.

Variety	Treatment	Percentage moisture fresh weight		Osmotic pressure in atmospheres		Pressure in lbs. (penetrometer)	Per cent solids
		50° C. in vacuo 72 hr.	100° C. 24 hr.	Cryoscopic method	Plasmolytic method		
Golden Russet	Controls	80 85	80 75	24.28	28.02	20 6	16 0
	5% CO₂	81 88	84.31	23 50	25 23	25.0	16 0
	10% CO₂	82.14	85 14	22.36	23 89	23 7	15 6
Cox Orange	Controls	82.49	84 35	22.42	—	15.5	15 2
	2 5% O₂	82 96	85.48	22 72	—	20.5	14 4
	2.5% CO₂	83.10	85.85	19 37	—	16.5	13 8
	5.0% CO₂	82.98	84 76	21 53	—	16 8	13 2
	10 0% CO₂	82 80	85 26	21 88	—	17 3	13 4
McIntosh	Controls	86 67	88 02	15 34	17 40	14 4	11 6
	2.5% CO₂	86.44	88 83	15 94	—	13.1	10 7
	5 0% CO₂	86.46	88.25	16.57	16 77	12 1	11 0
	10 0% CO₂	86.66	89.02	15.73	16.77	12 2	10 7

With the exception of the Golden Russet controls it will be seen that
drying at 100° C. for 24 hours gives a consistently higher figure for moisture
content than that at 50° C. for 72 hours (Table 7).

There is a general correlation between moisture content, osmotic values,
and percentage of total solids from the varietal standpoint. It must be
pointed out that the relative humidity was higher in the treated samples
than it was in the control lots, and thus the latter are consistently lower in
water content and higher in the observed osmotic values. It was found
later that Golden Russet apples which had been stored in air, one lot in
100% relative humidity, and another in 85%, showed osmotic pressures of
21.88 and 23.22 atmospheres respectively.

The difference between the osmotic pressures as obtained by the two
methods is in keeping with the findings of other workers (2, 9).

The pressure in pounds required to puncture the sub-epidermal tissues
of the fruit has been included in order to show in particular the remarkably
firm condition of the Cox Orange fruits stored in 2.5% oxygen in relation
to the high values obtained for the osmotic pressure, water content and
total solids. It would appear, therefore, that low oxygen conditions have
been instrumental in retarding the loss of cell wall materials, possibly
pectic constituents.

The effect of carbon dioxide would seem to tend toward decreasing
the osmotic pressure. The very low value obtained in the Cox Orange
apples treated with 2.5% carbon dioxide is attributed to the onset of
internal breakdown. A further attempt was made to elucidate the treat-
ment effects by calculating the osmotic values on the basis of the control
values for moisture.

The corrected values shown above again indicate that increased carbon
dioxide results in a lower osmotic value relative to the controls in air but
this point requires further investigation.

Determinations were first made on the permeability of apple cells to
various solutes, neutral red being used as the staining medium. The

TABLE 8.—OSMOTIC PRESSURES OF APPLE CELLS (CORRECTED FOR MOISTURE) STORED IN
DIFFERENT ATMOSPHERES FOR THREE MONTHS AT 3° C. TO 4.5° C.

Variety	Control in air	2 5% O$_2$	2 5% CO$_2$	5% CO$_2$	10% CO$_2$
Golden Russet	24.38	—	---	23 10	21.20
Cox Orange	22.42	22 6	19 20	21.75	21.75
McIntosh	15.34	—	16.05	16.65	15.75

following non-electrolytes were included: glycol, thiourea, urea, glycerol, and sucrose; also one electrolyte, namely, potassium nitrate. Sections were placed in twice isotonic solutions of the above substances and examined at 5-minute intervals for signs of deplasmolysis. It was found that the solutions of glycol and urea caused no plasmolysis, and there was only slight plasmolysis of the cells for 5 minutes with thiourea and glycerol. On the other hand, both sucrose and potassium nitrate produced strong plasmolysis in 5 minutes; the latter solute, however, was more powerful in its action, but neither of them showed signs of deplasmolysis at the end of 45 minutes after which time all the cells were dead. With the exception of thiourea it may therefore be concluded that the more polar the compound is the more impenetrable does the cell become to the same.

During the course of the above tests vacuolization was noted in carbon-dioxide-treated cells placed in thiourea and sucrose as shown in Figure 8. It is of interest to note that difficulty was experienced in obtaining these photographs as the vacuoles disappeared after a time. A peculiar characteristic which was observed in cells plasmolysed in a normal sucrose solution is shown in Figure 9. It would almost seem that there are two distinct membranes on the edge of the large vacuole in the centre cell; moreover, when the cell was observed prior to being photographed, the second or outer membrane was more pronounced and at a greater distance from the vacuole. There is the possibility, however, that in the process of plasmolysis a portion of the interior of the cell wall was drawn away with the protoplasm.

The relative permeability of cells to water in the case of treated Golden Russet apples was determined by measuring the time required for the cells to deplasmolyse. This was done by plasmolysing the cells in a twice isotonic solution of calcium chloride. They were left in this solution for about 20 minutes and then transferred to a half isotonic solution of the same salt for deplasmolysis. The average rate of permeability for 20 cells is shown in Table 9.

TABLE 9.—THE INFLUENCE OF CARBON DIOXIDE IN THE PERMEABILITY OF APPLE CELLS (3 MONTHS IN COMMON STORAGE, 3° C. to 4.5° C.) AVERAGE OF 20 CELLS

Treatment	Time required to reach incipient plasmolysis (min.)
Control in air	8
5% CO$_2$	6
10% CO$_2$	5

The figures given clearly indicate the increase in permeability of apple cells as a result of carbon dioxide treatment. This study was confined to the Golden Russet variety owing to sectioning difficulties with the other fruits. Nevertheless, in order to further substantiate these findings,

other studies were undertaken at the commencement of the next season with freshly-picked fruits. Samples of Gravenstein apples and Clapp Favourite pears were placed in containers, the atmospheres in which were as follows: 100% carbon dioxide, 100% nitrogen, and a control in air. Four temperatures were used: 0° C., 4.5° C., 10° C. and 18° C. A similar procedure to that described in the previous experiment was used.

TABLE 10.—THE INFLUENCE OF CARBON DIOXIDE AND NITROGEN ON THE PERMEABILITY OF THE CELLS OF APPLE AND PEAR FRUITS STORED FOR ONE WEEK AT °0 C., 4.5° C., 10° C. and 18° C.

Fruit	Temperature, degrees Centigrade	Time required to reach incipient plasmolysis (min.)		
		Control in air	100% CO_2	100% N_2
Pear	0	13.4	6.8	14.9
	4.5	7 7	9.0	12.0
	10	16 6	10.9	12.0
	18	12 5	12 0	7.0
Apple	10	15.0	7.0	20.0
Average for pears (all temperatures)		12.5	9.7	11.5

The results shown in Table 10 again indicate that carbon dioxide increases the permeability of cells to water; the results, however, with tissues stored in the absence of oxygen are not consistent. Temperature effects are not well defined although there is a consistent decrease in the permeability of the carbon dioxide series as the temperature is raised.

The viscosity of the apple and pear cells was tested by placing blocks of tissue in isotonic solutions and centrifuging them; sections were then taken from the tissue and examined as to the position of the starch grains. It was found that starch grains in the Gravenstein apple were thrown down too quickly (2 minutes) for timing purposes. The grains in the Clapp Favourite pears moved more slowly; accordingly, four samples of treated pears were tested as above; namely, in air, 100% carbon dioxide, and 100% nitrogen, each at 4.5° C., and 100% carbon dioxide at 18° C. This series was centrifuged for 5-minute intervals, in which it was observed that no movement of starch grains had taken place in the control and in the 100% nitrogen-treated apples, whereas in the carbon dioxide series there was a heavy accumulation of grains at the ends of the cells of tissues treated at 4.5° C., but only a slight movement had taken place in those tissues treated at 18° C. The temperature influence may be accounted for by the increased solubility of carbon dioxide at lower temperatures.

The permeability of apple tissues as measured by the diffusion of electrolytes into solutions of distilled water was studied in order to verify the foregoing data if possible. This was accomplished by placing discs of apple tissue in distilled water of a known electrical conductivity and measuring the decrease in resistance at 24-hour intervals.

Preliminary readings were made on uniform cylinders of tissue from apples which had been treated; the tissues from the 2.5 and 5% carbon dioxide treatments were affected with physiological breakdown and the increased leaching of ions from these cells is clearly evident (Table 11).

TABLE 11.—ELECTRICAL CONDUCTIVITY OF LEACHINGS FROM COX ORANGE APPLE TISSUES STORED IN ARTIFICIAL ATMOSPHERES (EXPRESSED AS RECIPROCAL OHMS $\times$ 10^5 AFTER 24 HOURS)

Variety	Control in air	Treatment			
		2.5% O_2	2.5% CO_2	5% CO_2	10% CO_2
Cox Orange	600	540	680*	666*	600

* Apples affected with internal breakdown.

The figures do not indicate a carbon dioxide effect when the controls and 10% carbon dioxide are compared, but the low permeability of the tissues stored in 2.5% oxygen is striking, also that obtaining in diseased tissue.

In view of the above results another experiment with Golden Russet apple tissue was set up in triplicate. Cylinders of tissue were removed from the apples by means of a cork borer (0.65 cm. bore) and cut up into discs 0.25 cm. thick. Discs of each sample were then placed in 50 cc. of distilled water, the conductivity of which was determined and found to be uniform. Three readings were made at 24-hour intervals, the fourth being discarded on account of mould development.

TABLE 12.—ELECTRICAL CONDUCTIVITY OF LEACHINGS FROM GOLDEN RUSSET APPLE TISSUE STORED IN ARTIFICIAL ATMOSPHERES FOR FOUR MONTHS (EXPRESSED AS RECIPROCAL OHMS $\times$ 10^5)

Variety	Time in hours	Serial number	Treatment		
			Control in air	5% CO_2	10% CO_2
Golden Russet	24	1	125	113	126
	24	2	126	113	126
	24	3	Lost	113	126
	48	1	145	156	178
	48	2	145	154	180
	48	3	—	153	180
	96	1	166	178	200
	96	2	166	180	200
	96	3	—	180	200

The permeability as shown in Table 12 is apparently increased by the carbon dioxide effect when the later conductivity measurements are compared. The first reading after 24 hours may be affected by the initial wounding of the tissues because thereafter the conductivity of leachings from sample to sample is quite consistent.

Fungal Activity

Two examinations of the stored fruits were made in order to determine the wastage due to fungal decay. The total decay for all varieties under each treatment is shown in Figure 7, the inhibitory effect of the artificial atmospheres employed being clearly demonstrated, particularly in 10% carbon dioxide. Examination of the data reveals, however, that the retarding influence of carbon dioxide and low oxygen is more marked in the early stages of storage as shown in Table 13.

TABLE 13.—PERCENTAGE OF FUNGAL DEVELOPMENT ON APPLES STORED IN DIFFERENT ATMOSPHERES FOR THREE MONTHS AT 3° C. TO 4.5° C.

Variety	Treatment	Examined		Ratio
		25/1/35	1/4/36	
Cox Orange (1)	Control in air	18.0	31.5	1.72
Cox Orange (2)	Control in air	16 0	60.0	3.74
McIntosh	Control in air	42.0	63.6	1.54
Cox Orange (1)	10% CO₂	2.0	26.3	13.50
Cox Orange (2)	10% CO₂	2.0	17.4	8.20
McIntosh	10% CO₂	0.0	20.0	20.00
Cox Orange (1)	2.5% O₂	10.0	24.0	2.40
Cox Orange (2)	2.5% O₂	4.0	44.0	11.00

In order to determine the influence of these same atmospheres upon the pure cultures of *Penicillium expansum* (the organism almost entirely responsible for the wastage observed in these experiments), isolations were made from decayed fruits and transferred to slants of sterile media (potato dextrose agar). After about two weeks the slants were examined and transfers were made from the pure cultures to petri plates (three for each treatment); these were immediately placed in the storage containers and the lids of the dishes removed. The control containers were covered with cheese cloth in order to eliminate contamination from the outside air as far as possible without interfering with the atmospheric conditions.

First of all, however, preliminary tests were made with sterile plates which were placed in the various atmospheres for 6 days in order to ascertain the degree of contamination to be found under such conditions. It is clear from an examination of Figure 10 that the infectivity of spores is considerably reduced by carbon dioxide and to a lesser extent by low oxygen. The influence of these same atmospheres on pure cultures of *Penicillium expansum* for a period of 12 days is shown in Figure 11. The following observations were made.

The control in air was characterized by heavier sporulation than was seen in all the other treatments, but growth was much the same as in 2.5 and 5.0% carbon dioxide; it is possible, therefore, that in the case of the controls, growth was retarded by the sporulating activities of the colony.

The colonies in 10% carbon dioxide and 2.5% oxygen showed less growth than in all other treatments and also less sporulation. In addition, the colony in 2.5% oxygen was peculiar in that aerial white mycelium developed in contrast to the depressed appearance of the other colonies.

Observations were also made after the plates were removed to room
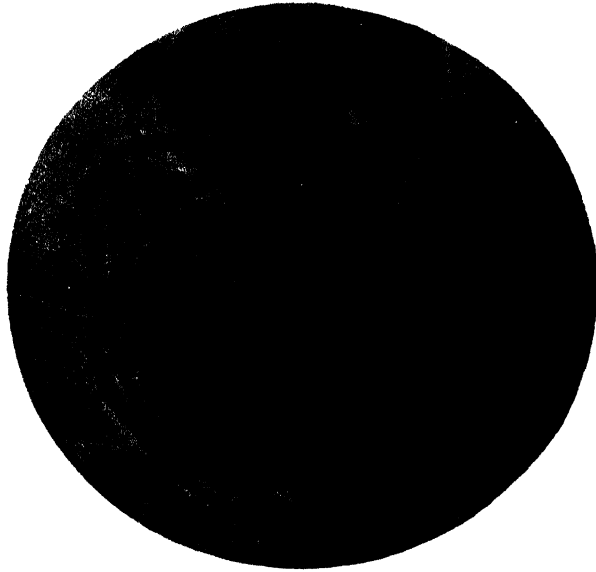
FIGURE 8. Plasmolyzed apple cells showing vacuolization (stained with neutral red).
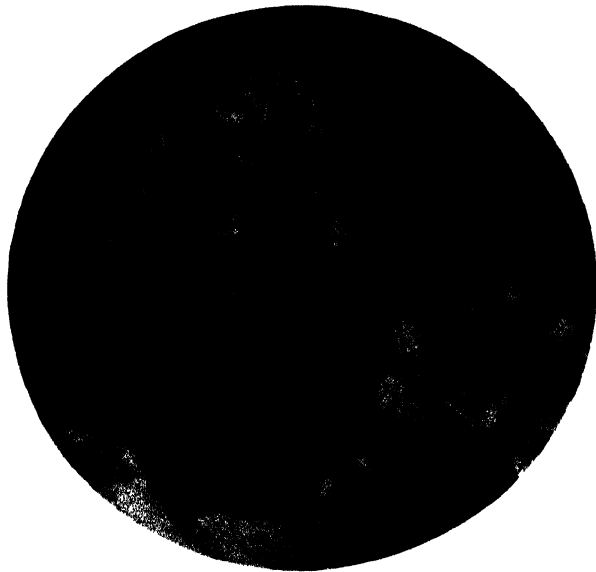
FIGURE 9. Plasmolyzed apple cells showing "double membrane" effect (stained with neutral red).
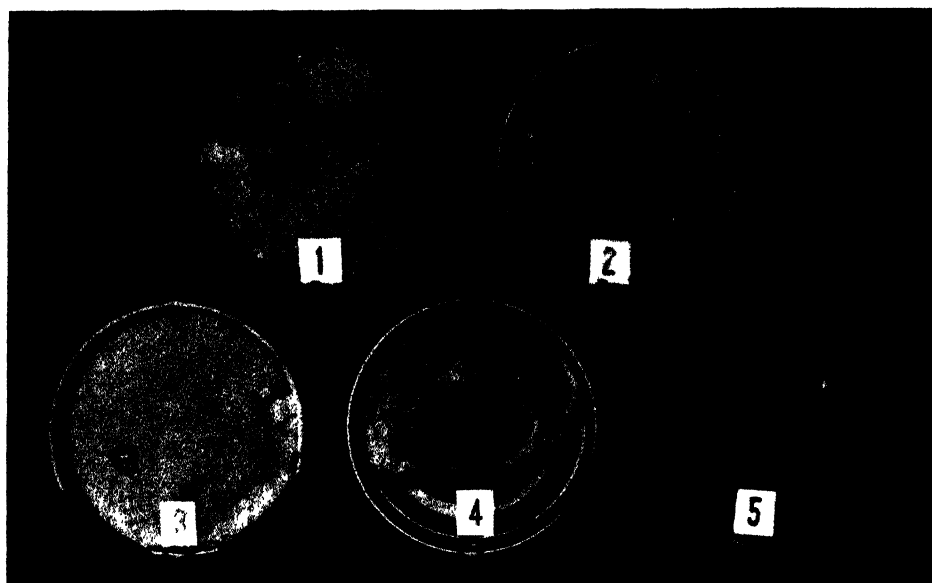
FIGURE 10. The influence of artificial atmospheres upon the growth of fungal spores on sterile media (Petri plates placed in apple storage containers). 1. Stored in air; 2. 2 5% O_2; 3. 2 5% CO_2; 4. 5 0% CO_2; 5. 10 0% CO_2.
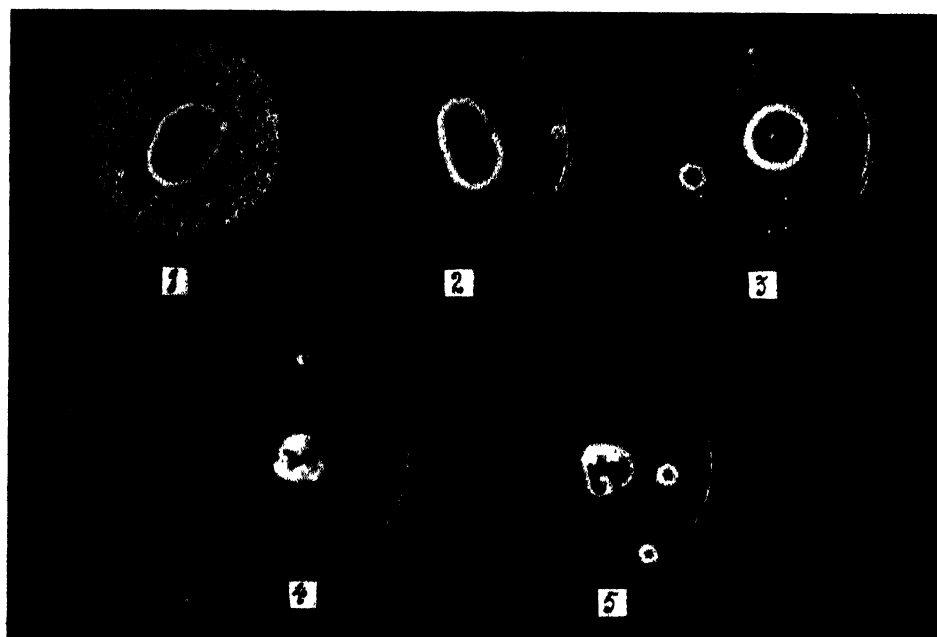
FIGURE 11. The influence of artificial atmospheres upon the growth of colonies of *Penicillium expansum*. 1. Stored in air; 2. 2.5% CO_2; 3. 5 0% CO_2; 4. 10.0% CO_2; 5. 2.5% O_2.
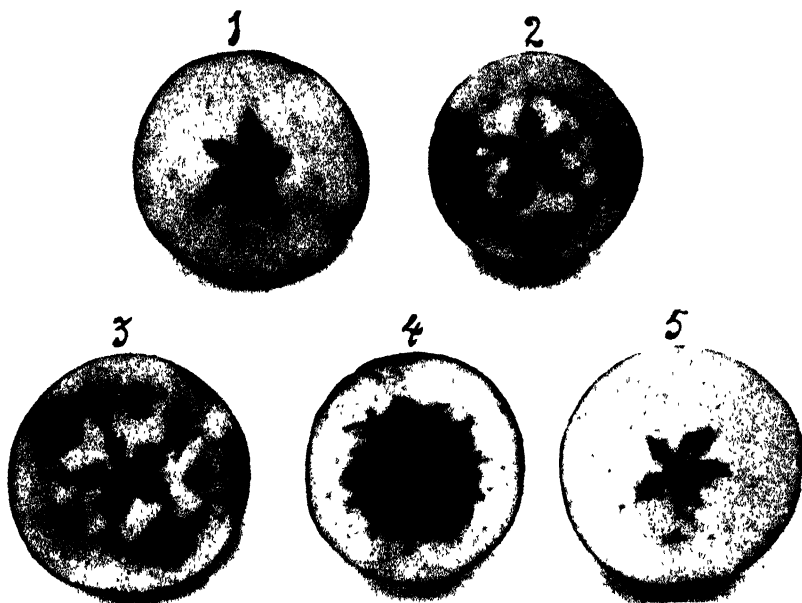
FIGURE 12. Physiological breakdown of Cox Orange apples stored in artificial atmospheres at 3° C. to 4·5° C. 1. Stored in air; 2. 2·5% CO_2; 3. 5·0% CO_2; 4. 10·0% CO_2; 5. 2·5% O.
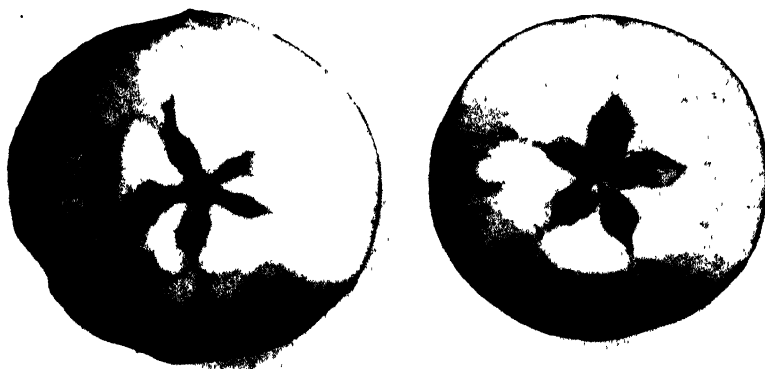
FIGURE 13. Physiological breakdown of Cox Orange apples stored in 2·5% carbon dioxide at 3° C. to 4·5° C.
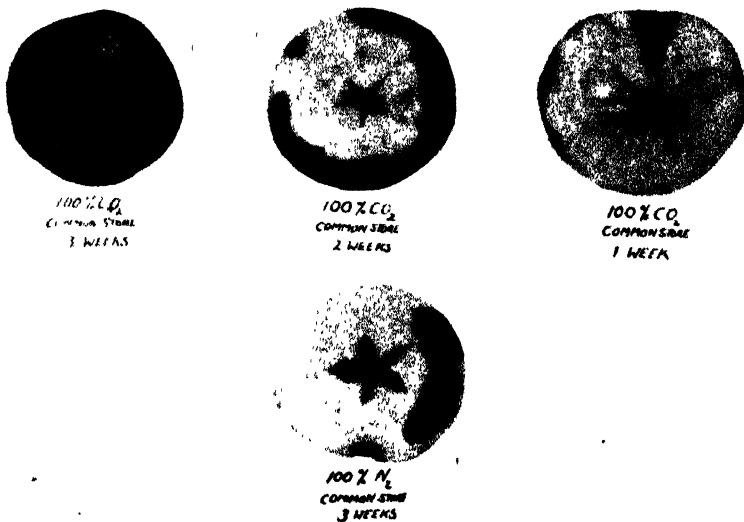
FIGURE 14. Physiological breakdown of McIntosh apples stored in 100% carbon dioxide and 100% nitrogen.
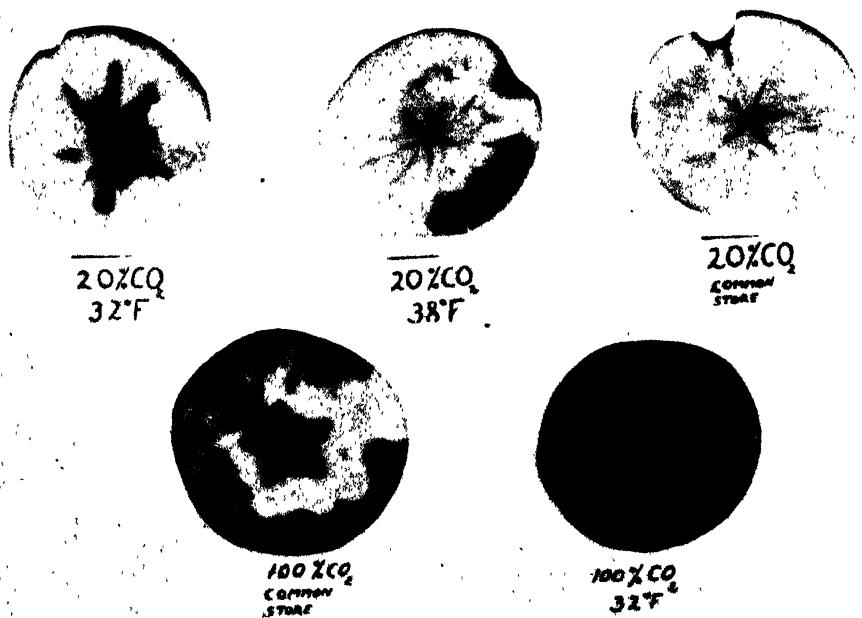
FIGURE 15. The influence of temperature on the breakdown of McIntosh apples stored in 20% carbon dioxide.

temperatures in air and it was seen that the control rapidly increased growth and sporulating activities as did the colony stored in 2.5% oxygen. On the other hand, the carbon dioxide treated cultures showed a slow reaction to environment, particularly that in 10% carbon dioxide, which might possibly indicate that the media had become toxic as a result of the atmospheric conditions during treatment.

Physiological Disorders

McIntosh apples stored in 10% carbon dioxide possessed a very pronounced alcoholic flavour, the samples in 5% concentration were slightly off flavour but those in 2.5% concentration remained in excellent condition. The low average temperature (3° C.) during the latter part of the storage season may account for the flavour developed in the sample stored in 5% carbon dioxide. No wastage due to functional disorders was observed with this variety with the exception of a slight corticle flush which appeared in the controls in air. Golden Russet apples remained in perfection condition in both 5 and 10% carbon dioxide concentrations until the sixth month when it was observed that a mottled type of scald had made its appearance. In the 5% carbon dioxide treatment, 77% of the apples were scalded, whereas in the 10% series only 31% were affected. The necessity of wrapping fruits of this variety in oiled paper for storage under such conditions at once becomes apparent. The controls in air were only characterized by heavy shrinkage. Duplicate samples of all treatments in the Cox Orange series were carried in storage. Table 14 shows the results obtained.

TABLE 14.—FUNCTIONAL DISORDERS AND FLAVOUR OF COX ORANGE APPLES STORED IN ARTIFICIAL ATMOSPHERES IN COMMON STORAGE (3° C. TO 4 5° C.)

Serial number	Per cent CO_2	Per cent O_2	Per cent breakdown Examined		Flavour 25/1/36
			25/1/36	13/4/36	
14	0 0*	2 5	—	—	Slightly bitter
15	0.0*	2.5	—	—	Slightly bitter
12	2 5	18.5	20.0	21.0	Good
13	2 5	18.5	10.0	50.0	Good
8	5 0	16.0	22.0 (heavy)	72.7	Good
9	5 0	16.0	2.0	100.0	Good
10	10.0	11.0	—	47 4	Slightly alcoholic
11	10 0	11.0	—	35 5	Slightly alcoholic
Control	—	—	—	—	Mealy (No flavour)

* Determined.

Table 14 indicates that the onset of functional disorders is induced by increased carbon dioxide concentrations, and that apart from deleterious effects upon the flavour the low oxygen treatment and the controls constituted the best treatments (see Figure 12). The low oxygen concentration, furthermore, is characterised by very hard firm fruits which corresponds with observations made by the writer on pears treated in a

similar manner. The relatively high percentage of breakdown in the 5% carbon dioxide series is particularly striking. The appearance of the breakdown as found in each treatment is shown in Figure 12 and it would seem that the physiological effects are more or less specific. In Figure 13 it will be seen that the disorder in apples stored in 2.5% carbon dioxide is confined to the peripheral tissues, a characteristic of mealy breakdown. Furthermore, Table 14 shows that breakdown in the first examination was confined to the 2.5% and 5% series. The latter samples were not so markedly affected in the peripheral areas but rather more so in the vascular areas. The apples stored in 10% carbon dioxide are in contrast to the above treatments in that disintegration of the tissues is more or less limited to the core area including the 10 main vascular bundles.

Careful comparison with the illustrations of mealy breakdown and brown heart as shown in the publication by Plagge and his associates (28) would indicate that the 2.5 and 5% treatments are types of mealy breakdown, while 10% carbon dioxide has induced brown heart.

Observations on the effects of atmospheres devoid of oxygen upon McIntosh and Stark apples at room temperature after storage at 3° C. for 4 months showed that both varieties developed breakdown which resembled soggy breakdown. This type has been previously noted at the Kentville Experimental Station in McIntosh apples exposed to 100% nitrogen at 0° C. for 3 weeks after 2 months storage, but did not appear until a month later in common storage (see Figure 14). The disorder usually arises in the corticle region of the apple as scattered light brown patches. At first these are well defined, but they gradually merge into a band-like area of soft tissue, approximately 0.5 cm. from the epidermis, as seen when an affected apple is halved at right angles to the core line. This band of dead tissue may or may not completely surround the cortex, but in the advanced stages of development the healthy sub-epidermal tissue gradually breaks down, which is followed by external browning of the apple skin and final disintegration of the entire fruit. As will be noted from the above table, low oxygen conditions produced a bitter flavour in the fruit and which was more marked in apples stored in pure nitrogen immediately after harvesting for a period of one week.

Finally the effects of high concentrations of carbon dioxide upon McIntosh apples are shown in Figures 14, 15; it was noted that very pronounced alcoholic flavours developed in the fruits and that the degree of tolerance toward the gas was less at low temperatures.

DISCUSSION

These investigations have been directed toward a consideration of the physiological effects of artificial atmospheres upon the cellular activities of senescent fruits. It has been shown that the total carbon dioxide output of apples is depressed and stimulated by high (53.5%) and low (6.2%) concentrations of carbon dioxide, respectively, during a period of 5 days. It is necessary, however, to bear in mind the findings of Kidd, West and Kidd (21) who have shown that prolonged storage in 5 and 10% carbon dioxide depresses the respiratory activity of fruits. The development of alcoholic flavours under conditions of high carbon dioxide would point to a stimulation of the anaerobic fermentation system in the cell.

The increase in carbon dioxide output of fruits which were removed from air to pure nitrogen may be due in part to the expulsion of carbon dioxide within the tissues as suggested by Gustafson (14) and to the protective role of aerobiosis in relation to anaerobiosis as postulated by Blackman (3). The corresponding increase in carbon dioxide evolution from apples when removed from high concentrations of the same gas to air also indicates a rapid expulsion of carbon dioxide and possibly the temporary nature of the narcotic influence exerted by carbon dioxide (12, 33). Further the oxygen uptake of the nitrogen-treated fruits when returned to air was lower than that which obtained in those stored in air in spite of the greater carbon dioxide output of the former. It would appear, therefore, that anaerobic processes were still functioning in the treated fruits when stored in air. No evidence was obtained however regarding the extinction point (E) of fermentation in these fruits (31).

The respiratory activity of fruits was determined in air at 3° C. and at 21° C.; the apples used had been stored in 5 and 10% carbon dioxide for approximately 4 months. It was observed that the treated apples evolved a greater amount of carbon dioxide than the controls upon removal to 21° C. The higher value may be due first to the higher concentration of respirable substrate, and second to the disappearance of accumulated carbon dioxide within the tissues. The first explanation is of interest when it is remembered that the Golden Russet apples stored in a relative humidity of 100% in air evolved as much or more carbon dioxide than did the treated fruits. The importance of moisture relationships at once becomes apparent, and in this connection it was found that apples of the same variety lost approximately three times as much moisture as carbon dioxide. In contrast Wardlaw and Leonard (37) report that tropical fruits lose 10 times as much water as carbon dioxide. It was found that the loss of these constituents from apples varied inversely as the size.

A marked deceleration in the rate of loss of total acids in fruits stored in carbon dioxide was observed. Magness and Diehl (24) found that acid loss increased in Delicious and Winesap apples stored in carbon dioxide. Kidd, West and Kidd (21) on the other hand showed that the acid loss was retarded in Bramley's Seedling apples stored in 10% carbon dioxide.

Juice from apples stored in low concentrations of carbon dioxide for 3 months was characterized by a lower pH value than that obtained with juice from air-stored fruits; a higher pH value was obtained, however, when apples were subjected to 100% carbon dioxide for one week. Nevertheless, Thornton (35) states that carbon dioxide in the presence of oxygen decreases the hydrogen ion concentration in a wide range of plant tissue including the McIntosh apple, both in high and low concentrations of carbon dioxide at 25° C. On the other hand, potato tubers treated with 100% carbon dioxide increased in acidity. No significant changes were found in the pH values of cherries, plums and peaches which had been treated with 50% carbon dioxide according to Miller and Dowd (25). Jacobs (17) has shown that carbon dioxide has a specific effect as an undissociated gas producing lethal effects at an increased hydrogen ion concentration.

Pure nitrogen and low oxygen treatments had little effect upon the hydrogen ion concentration of apple juice although there was a slight tendency toward a less acid reaction. Fidler (11) has shown that rate of acid

loss in apples and oranges remains unaffected by the absence of oxygen. He suggests that acid loss is not due to oxidation to carbon dioxide but rather that it undergoes reduction with the evolution of oxygen which could in turn oxidize some intermediate product of carbohydrate metabolism.

Loss of carbohydrates as total sugars appeared to be increased by storage in both 10% carbon dioxide and 2.5% oxygen; this was more marked in the sucrose fraction, particularly under the latter conditions. These findings are in accord with those of Kidd, West and Kidd (21) in regard to the carbon dioxide effect, and with those of Fidler (11) who found that sucrose is lost more rapidly under anaerobic conditions.

The data obtained on osmotic values were complicated by the presence of lower relative humidities in the control containers than in those containing artificial atmospheres. Nevertheless the carbon dioxide treatments appeared to reduce, and the low oxygen to increase, the osmotic pressure in apple cells relative to the controls in air. There was found to be a varietal correlation between osmotic pressure, water content and the percentage total solids in the juice. That there is a wide variation in the osmotic values of cells in different varieties of apples is supported by the work of Chandler (7). Walter (36) lists the following fruits together with the respective osmotic pressures in atmospheres: apples, 23.81; grapes, 28.0; tomatoes, 8.79; and citrus fruits, 14.0.

It has been shown that carbon dioxide increases the permeability of the cells both to water and to leachings. Furthermore, apolar compounds such as urea, penetrate into the cell, while calcium chloride and potassium nitrate, which are strongly polar, failed to enter. These findings are in line with the work of Jacobs (19) who observed that the penetrability of solutes is a function of lipoid solubility supports the hypothesis of Overton (27) that the plasma membrane of the cells is composed largely of water with a differential lipoid phase. Heilbrunn (16) points out that carbon dioxide is frequently used as an anaesthetic in physiological studies and thus may cause dissolution of the lipoid phase (a characteristic of anaesthetics). This observation is borne out by the findings of Jacobs (18) who noted that with *Spirogyra* short exposures of carbon dioxide brought about a liquefaction of the protoplasm while long exposures caused coagulation. In studies on the carbon dioxide narcosis of *Nitella*, Fox (12) observed an increase in the permeability of cells, vacuolization and cyclosis or stoppage of protoplasmic streaming which he suggests is due to the gelation of the colloidal matrix as seen by the jerky motions of the granules (unlike Brownian movement) caused by the kinoplasm (or streaming plasm) when the granules become fixed in the gel.

No consistent differences between the fruit held in low oxygen and in air were noted in relation to water permeability.

Several investigations have shown that carbon dioxide produces an inhibitory influence upon the growth of fungal organisms (4, 5, 6). These studies further substantiate these findings; fungal activity was definitely retarded by 10% carbon dioxide concentrations and to a lesser extent by lower concentrations of the same gas and 2.5% oxygen. This inhibitory influence, however, appeared to lessen in the later stages of storage. Pure cultures of *Penicillium expansum* exposed to similar treatments were also

retarded in growth and sporulation was reduced. It was observed that the colonies subjected to 10% carbon dioxide for 9 days and then placed in air did not exhibit the rapid recovery that was shown by the colonies stored under low oxygen conditions. This continued growth retardation of the carbon-dioxide-treated colonies when removed to air may have been due to the acidification of the media or to the alkaline reaction of fungal hyphae as shown by Thornton (34).

The final stages of the senescent activities of fruit are characterized by physiological breakdown of the tissues, but a detached analysis of these activities is rendered extremely complex due to the inherent varietal reactions of fruits to environmental influences both before and after removal from the tree. The chemical studies on the physiology of apples by Archbold (1), also those of her colleagues, are invaluable contributions in this field of investigation.

It has been found in these studies that physiological breakdown of the apple is associated with marked increases in the respiratory activity, transpiration, permeability and the alkalinity of cells so affected. The accelerated loss of sugars, as seen with apples treated with low oxygen concentrations, did not appear to induce disorganization of the cells; indeed these fruits were shown to have relatively high osmotic pressures, percentage of total solids and moisture content. Furthermore, if it be assumed that the strength of the cells can be gauged by the amount of pressure required to break the same with a penetrometer, then these cells which were subjected to low concentrations of carbon dioxide and also oxygen are high in cell wall materials such as pectins and cellulose.

From the evidence obtained, it is apparent that the substrate for respiration must consist not only of the carbohydrates in the cell but also certain cell wall constituents, because in atmospheres in which there is a low rate of oxidation the disappearance of pectic materials is apparently retarded. Furthermore, it is thought that the supply of acid is not necessarily limited by the amount of sucrose present.

The increase in the permeability of carbon-dioxide-treated (2.5 and 5%) cells may possibly be attributed to the dilution of protoplasmic colloids, in view of the relatively high water content of these tissues; this is partially supported by the relatively high respiration rates of turgid fruits. The only explanation that can be offered for this increase in permeability, which is characteristic of physiological disturbances, is that it may be offset by the slow rate of oxidation under such conditions.

The breakdown occurring under the above conditions has been designated as "mealy breakdown" and is possibly due to the combined effect of low temperatures and carbon dioxide.

Excessive concentrations of carbon dioxide have been shown to induce the condition known as "brown heart" which is usually initiated in the core area of senescent fruits where the internal concentration of carbon dioxide is relatively high. This disease has been described in detail by Kidd and West (22).

Fruits which had been stored for a prolonged period at 3° C. were found to break down after exposure to 100% nitrogen at 21° C.; there was also a loss of red pigmentation in these apples. No such disturbances were noted, however, in the freshly picked apples under similar conditions.

It is possible, therefore, that these physiological responses in the senescent fruits were due to the run-down state of the enzymatic system and its consequent inability to withstand withdrawal of the oxygen supply.

The superficial scald on Golden Russet apples which appears very late in the storage season in carbon dioxide is difficult to explain. It may be that the extinction point (E) for these fruits is reached at the time of scald development and that some product of anaerobic fermentation, as, for example, acetaldehyde, proved toxic to the epidermal cells. Davis and Blair (8) recommend the use of oiled wraps for the prevention of scald in apples stored in artificial atmospheres.

In conclusion, we have seen that storage in carbon dioxide produces certain cellular changes typical of undesirable physiological conditions within the cell. It is believed, however, that the slow rate of oxidation of cell wall materials in low concentrations of carbon dioxide is of considerable importance in the longevity of the cell. Furthermore, the retardation of acid loss by carbon dioxide below a certain critical concentration is possibly responsible for a high degree of peptization being maintained in the protoplasmic colloids, a condition which prevents dehydration and final coagulation of the protoplasm.

SUMMARY

The influence of carbon dioxide and low oxygen concentration on the physiological activities of apple tissue has been studied.

It has been shown that low (6.2%) and high (53.5%) concentrations of carbon dioxide stimulate and depress respectively the total carbon dioxide output of fruits during a period of 5 days. Low concentrations of oxygen (1%) increased the output of carbon dioxide of apples; when these fruits were returned to air the rate of oxygen uptake was found to be lower than in those fruits stored continuously in air.

Fruits which had been stored in 5% and 10% carbon dioxide at 3.5° C. for several months and removed to 21° C. were observed to respire more rapidly than the controls stored in air.

Moisture loss and the size effect were noted. Golden Russet apples lost 3 times as much moisture as carbon dioxide by weight, and the loss of these constituents from fruits varied inversely as the size. In the fruits which were treated with carbon dioxide (2.5, 5 and 10%), over long periods there was found to be an increased hydrogen ion concentration in relation to the controls in air. A decrease in hydrogen ion concentration of apple and pear juice was observed in fruits stored in 100% carbon dioxide for one week and this was more marked at high temperatures; a slight increase was also seen with fruits kept in 100% nitrogen.

Carbohydrate loss appeared to be accelerated by carbon dioxide treatment, and an increased loss of sucrose in the low oxygen series was evident, but a definite retardation was noted in the loss of total solids in the expressed juice of fruit under the latter conditions as compared with the former.

Apple cells were found to be more permeable to apolar compounds than to polar, and carbon dioxide increased the permeability of cells to water, particularly at low temperatures, as measured plasmolytically. The electrical conductivity of leachings (electrolytes) from tissues immersed

in distilled water indicated that carbon dioxide increased the permeability of cells, but that low oxygen caused a decrease.

Fungal invasion was retarded by carbon dioxide and to a lesser extent by low oxygen.   Colonies of *Penicillium expansum* were likewise retarded both in growth and sporulation.

Two types of physiological breakdown in Cox Orange apples were observed; namely, "brown heart," due to excessive carbon dioxide concentration (10%), and a condition similar to "mealy breakdown" in the 2.5 and 5% treatments.   A type of breakdown and loss of pigmentation occurring in McIntosh and Stark apples stored in 100% nitrogen is described. Lastly, it was noted that Golden Russet apples developed superficial scald when stored for prolonged periods in carbon dioxide.

Finally, fruits affected with physiological disorders were found to have the following characteristics: (1) high rate of respiration, (2) high rate of moisture loss, (3) low acid content, (4) low total solids, (5) low osmotic pressures, and (6) increased permeability to water and electrolytes.

ACKNOWLEDGMENTS

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"ICES"

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Numerous enquiries made at recent fishermen's meetings and instruction courses, concerning the relative cooling capacities of various water ices, gave rise to the simple experiments about to be described. Although most of the enquiries were answered at the time by reference to the general principles of physics and refrigeration, it was considered that actual experiments should be subsequently conducted to make the arguments more convincing, because it was realized that theoretical discussions are often regarded with skepticism by the practical man.

The fishermen are the ones who use the ice and their needs are of first importance when it comes to considering the ice required for the proper preservation of their catches.   So it is important that the ice manufacturer study these needs and attempt to fulfill them to the best of his ability commensurate with modern equipment, economy and most recent knowledge of the preservation of fresh fish at sea.

Fishermen often complain that they are sold what they call "green" ice; that is, ice just taken from the freezing tanks. This is a just complaint, because the outside of blocks of ice straight from the freezing cans must naturally be wet due to the thawing necessary to extract the blocks from the cans.   Therefore when such blocks are immediately crushed and discharged into the hold of a vessel, wet particles of ice become intimately mixed with particles having a temperature well below the freezing point, and the result is a solid frozen mass when the fisherman subsequently comes to ice down his catch.   Under such circumstances it is well nigh impossible to make a satisfactory job of icing down the fish; consequently fishermen have come to the conclusion that ice is improved by long storage and they object to the use of ice that has not been stored for some time.

Actually, as table II will show, the period of storage has no effect upon the cooling capacity of ice, once equilibrium with its surroundings has been reached.   What is of importance, however, is the temperature of the ice as it enters the hold.   The lower this temperature, the greater is the cooling capacity of the ice and the greater are its chances for remaining free-running in the hold; that is, the drier and more powdery it will remain, except for a frozen layer on the top and around the edges of the pile where warm air comes in contact with it.   Consequently it is not essential to store ice longer than is necessary to refreeze the outer wet surface of the blocks and let the mass come to the temperature of the storage room.

There is some advantage to the fisherman, however, if the temperature of storage is maintained moderately low, because as already mentioned not only does the ice remain free-running and more convenient to handle, but each 3° F. lowering of the storage temperature adds approximately 1% to the cooling capacity of the ice.   Although it may not be economically feasible to lower the storage temperature a great deal, in

existing plants where the ice run-ways and crusher rooms are not refrigerated, the initial temperature of the ice should be sufficiently low to assure that the ice gets to the hold of the vessel in a dry state to guard against subsequent freezing together of the individual lumps or flakes.

To show the effect of storage temperature upon the cooling capacity of ice the following table has been included:

TABLE I—Effect of Temperature of Ice on Its Refrigerating Ability

Temp. of crushed ice, as it enters the hold	Heat absorbed to raise temp. of ice to melting pt. (B.t.u. per lb)	Heat required to melt ice (B.t.u. per lb)	Per cent refrigeration added due to ice being below its freezing point
32° F.	0	144	0
28°	2	144	1.4
20°	6	144	4.2
10°	11	144	6.9
0°	16	144	11.1
–10°	21	144	14.6

In compiling this table the specific heat of ice—that is, the heat required to raise the temperature of 1lb of ice 1° F.— was taken as 0.50 B.t.u. per lb. Actually the specific heat varies from 0.493 B.t.u. per lb at 32° F. to 0.435 B.t.u. per lb at –40° F., where the B.t.u. (British thermal unit) is 1/180 of the heat required to raise the temperature of 1lb of water from its freezing point (32° F.) to its boiling point (212° F.), or roughly, the amount of heat required to raise the temperature of 1lb of water 1° F. or the amount of heat given up in cooling 1¼lb of halibut 1° F.

Apart from the questions of "green" and aged ice and the temperature of storage, questions have also been asked about the comparative cooling capacities of natural ice, snow, very finely crushed ice, coarse ice and benzoic acid ice. These were all tested for their latent heat of fusion— that is, the quantity of heat required to change 1 lb of ice or snow to water at 32° F.—with the result given in table II. The standard method of mixtures was employed for these determinations and the results given are the means of numerous trials.

It will be seen that with the exception of frost taken from the coils in one of the storage rooms and snow, there is practically no difference between the various ices tested. Some fishermen have expressed the opinion that natural ice is superior to manufactured ice. This is very doubtful in the light of the above results, because natural ice could not exceed distilled water ice in its cooling capacity and the results for distilled water ice are not significantly higher than those for the manufactured ices. It may be assumed, therefore, that there is no practical difference in the latent heat of fusion or what is popularly called "cooling capacity" of the ordinary commercial water ices.

Somewhat lower results were obtained for the frost from the coils and snow, but even here the divergencies are insignificant from a practical point of view and may be disregarded.

TABLE II—Heat Absorbed (Refrigeration) Due to Melting of Different Kinds of Ices

Kind if ice	Latent heat of fusion (B.t.u. per ℔)
Tap water ice (clear)	143.3
Tap water ice (white)	143.5
Clear ice 3 months old	143.5
Clear ice 1 day old ("green")	143.5
Chunk ice	143.5
Pulverized ice	143.5
Distilled water ice	143.6
Snow	142.7
Frost from coils	142.6
Benzoic acid ice	143.3

The above results also show the unsoundness of the view that, because coarsely crushed ice lasts longer in the hold than finely crushed ice, it is better for cooling purposes. If the more finely crushed ice disappears faster in the hold it indicates that it is absorbing heat more rapidly and therefore must be inducing a lower temperature, thereby serving its purpose better than the coarse ice would.

To further investigate this question of the relative temperature effects induced by chunk ice and finely crushed ice, the two kinds of ice were tested simultaneously in two adjacent air tunnels through which air was circulated at a constant rate. The same weights of ice were placed in each tunnel. In tunnel C the ice was in large chunks and in tunnel F the ice was comparatively finely crushed. Figure 1 shows the temperature of the air entering the tunnels and also the temperature of the exit air from each tunnel.

It will be noticed that for over 2 hours the temperature of the air in tunnel F remained from 5° F. to 1.5° F. below that in tunnel C, because of the greater surface area exposed to the passing air. At the end of approximately 3 hours both tunnels were at the same temperature, showing that by this time roughly the same area of ice was exposed in both. After this the temperature of the air emerging from tunnel F rose about 2° F. above that in tunnel C. At the end of 7 hours all the fine ice had disappeared and only 11% of the coarse ice remained.

Since the object of taking ice on fishing trips is to preserve the fish, not the ice, and it is recognized that the lower the temperature the better the fish keep so long as freezing does not take place, the above results indicate that the finely crushed ice is better than coarse ice for preserving the fish.

Experiments similar to the above were conducted to compare some of the ices mentioned in table II for the temperatures induced and their lasting quality in melting tests. Distilled water ice was compared with tap water ice; freshly made ice with stored ice; clear ice with white ice or ice made without air agitation; and ordinary ice with benzoic acid ice. In no case was a significant difference observed so long as the initial size and temperature of the ice specimens were the same in a given test.

It may be assumed, therefore, that any differences found by fishermen in the cooling capacity of ices from different manufacturers are due to the temperature of the ice entering the hold and the degree of fineness of the crushed ice.
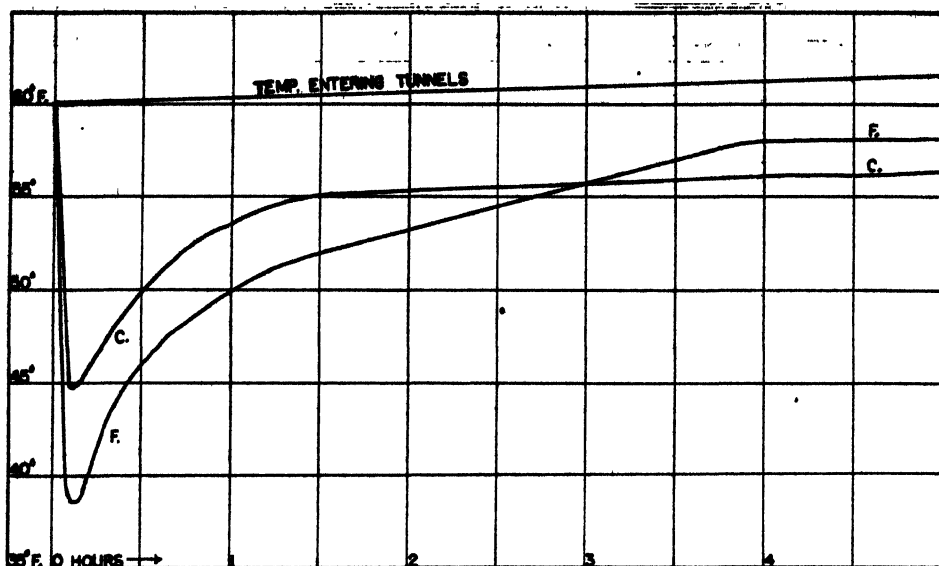
Fig. 1—Comparative temperatures produced by melting of coarse and fine ice.

Eutectic Ice

For maintaining temperatures several degrees above 32° F., so far water ice is generally accepted as satisfactory in most respects; but for maintaining temperatures below 32° F. no one refrigerant has yet received general acceptance.

The storage and transportation of frozen comestibles demand temperatures much below 32° F. and technical investigations indicate that not only are low temperatures desirable but constancy of temperature is also highly desirable.

The method most commonly used to obtain temperatures below 32° F. for short periods of time is to mix ice and salt in various proportions depending upon the temperature desired. Theoretically a temperature of –6° F. may be obtained with salt and ice in eutectic proportions, but in practice that temperature is rarely approached. One disadvantage of salt ice mixtures, therefore, is the difficulty of obtaining uniform temperatures throughout the mass, and consequently a constant temperature of the comestible cannot be obtained. A further disadvantage is the low heat absorbing capacity of the mixtures.

Eutectic salt ice overcomes these two main disadvantages. First it has a constant melting point of approximately –6° F. and secondly it has a much higher heat absorbing capacity. The heat required to melt 1 ℔ of eutectic salt ice at –6° F. is stated to be approximately 102 B.t.u.,* whereas the heat required to melt 1 ℔ of a eutectic mixture of salt and ice was found in these laboratories to be about 83 B.t.u. It will be seen therefore, that eutectic salt ice is much superior to salt ice mixtures where a low constant temperature is desired.

* Field and Adams, Refrigerating Engineering, February, 1936.

Studies on Salt Fish

III. Equilibrium Moisture Coefficients of Salt Fish Muscle

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ABSTRACT

Sorption isotherms of salt fish muscle determined by the quartz spiral balance show that the material is isohygrometric between 0.30 and 0.754 activity and 15° to 35°C. temperature. Above 0.754 the system acts as a saturated solution of sodium chloride.

An attempt to treat the drying of salt fish muscle analytically disclosed that no data on the equilibrium between the muscle system and the pressure of vapour in the ambient air were available. These equilibria are usually called the equilibrium moisture coefficients of the material under treatment when drying is considered, or more generally the adsorption coefficients. The purpose of the work described herein was the determination of these coefficients for fish muscle containing approximately 50 per cent salt, and water vapour.

These equilibrium moisture coefficients are usually determined during concurrent experimentation on the rate of drying of the material, but this method has several objections which have been fully discussed by Pidgeon (1934a). This work was carried out therefore by using a McBain-Bakr adsorption balance of the familiar type in combination with a suitable arrangement to give constant vapour pressures variable at will between the required limits.

EXPERIMENTAL

APPARATUS

The apparatus is shown in figure 1. It consisted of (S) a water bath controlled in the usual manner, in which were placed two adsorption tubes connected to a mercury manometer (M). Samples were suspended on quartz spirals (C, C) hung from hooks on the inside of these bulbs. The apparatus was connected with a suitable vacuum system and a constant temperature bath (D, J) for controlling vapour pressure, (Pidgeon 1934b). A cooling coil (not shown) allowed the bath to be operated below room temperature. Experimentation could be carried out within the range 15° to 35°C.

Lengths of spirals, and pressure differences on the manometer, were read on a cathetometer with a vernier to 0.001 cm.

The quartz spirals were wound as described by Tapp (1932) and Pidgeon (1934c). These showed the usual straight line characteristics, their equations being

Spiral no.	Equation
3	$W = 0.010150\ E - 0.9622$
4	$W = 0.006696\ E - 0.061270$
5	$W = 0.021490\ E - 0.30787$

where W is the weight in grams, and E the extended length in cm.

ACCURACY

The constant temperature bath was maintained to 0.03°C. Comparison of the bath thermometer with standards agreed to within 0.02°C. Low vapour pressures could be maintained constant to within the limits of reading with the cathetometer. In practice it was found that a somewhat greater variation occurred at higher pressures, therefore both at low and high activities values of the latter are correct to within 1.3 per cent.
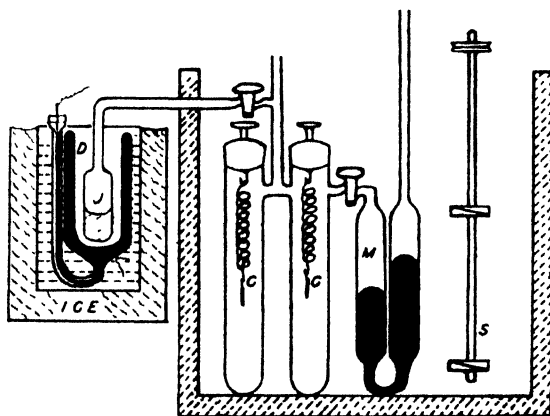
FIGURE 1. McBain-Bakr adsorption balance and Pidgeon control bath. D,—thermostat, J,—water tube, c, c,—spirals and samples, s,—stirrer.

Uncertainties due to weighing are approximately 3 per cent at low and 0.3 per cent at high activities, using samples of about 0.04 gm.

All samples were cut from fish containing approximately 50 per cent salt, known commercially as "hard salted". A number of samples were prepared in the laboratory using "C P" and various commercial brands of salt. Others were bought in the open market. The laboratory samples were prepared either by dry salting ("in kench") or by immersion in saturated salt solution ("pickle salting"). Descriptions of the methods are given by Huntsman (1927) and MacPherson (1932). The relation between the method of treatment and the nature of the product obtained is discussed by Rheay (1936).

Samples for examination were usually cut from the flesh at the thick part

of the nape. These were attached to the springs by small quartz hooks weighing about 0.004 gm. As a rule, all samples were dried to constant weight in vacuo and the equilibrium determined during increase. In one case the apparatus was evacuated without appreciable loss from the sample by the addition of a few drops of saturated salt solution to each tube, the last traces being distilled to the constant pressure tube and the equilibrium values determined during successive lowerings of the vapour pressure. This determination gave the downward curve shown in figure 2.

About 40 samples were treated, 6 at 15°C., 28 at 25°C., and the remainder at 35°C.

RESULTS

The results are shown in table I and figure 2.

TABLE 1

Activity	Equilibrium moisture contents	
	Downward	Return
0.20	. . .	2.7
0.30	. . .	3.7
0.40	5.7	5.0
0.50	7.7	6.7
0.60	12.0	9.1
0.70	24-25.5	19.4
0.725	30-35	27.6
0.75	45	. . .

Within the limits of experimental error the equilibrium moisture (per cent of dry weight) values for all samples were the same at equal activities. This was found to be true irrespective of change in temperature or in the nature of the sample. At high activities a definite hysteresis was observed.

Examination of figure 2 shows that below a = 0.76 salted fish muscle acts as a wettable surface for the adsorption of water vapour. Above this value the regain and falling rate curves coincide, and the equilibrium values become indeterminate. It appears, therefore, that above a = 0.753 to a = 0.754 the system acts as a saturated solution of NaCl, and distillation occurs on the sample until the activity of the water in the sample becomes equal to that in the surroundings. This view is supported by the fact that whenever the activity was raised above 0.76 visible drops formed on the samples, which on standing increased in size and eventually dropped to the bottom of the tubes.

The activity over saturated salt solutions remains constant, within the accuracy of these experiments, in the range 15° to 35°C. (Speranzi 1910).

Falling rate values and regain values differ as shown in figure 2. The upper section of the falling rate curve is uncertain and is therefore shown on broken lines. The accuracy of experimentation was not sufficient to distinguish between the two broken lines shown. Equilibrium during regain was usually more definite than that determined during the loss of moisture. This probably

.was a result of slight changes in vapour pressures at high values, the effect of which is discussed by Pidgeon (1934a).

The effect of the presence of air on this hysteresis is not known. There is however no *a priori* reason for assuming that air would cause a difference.
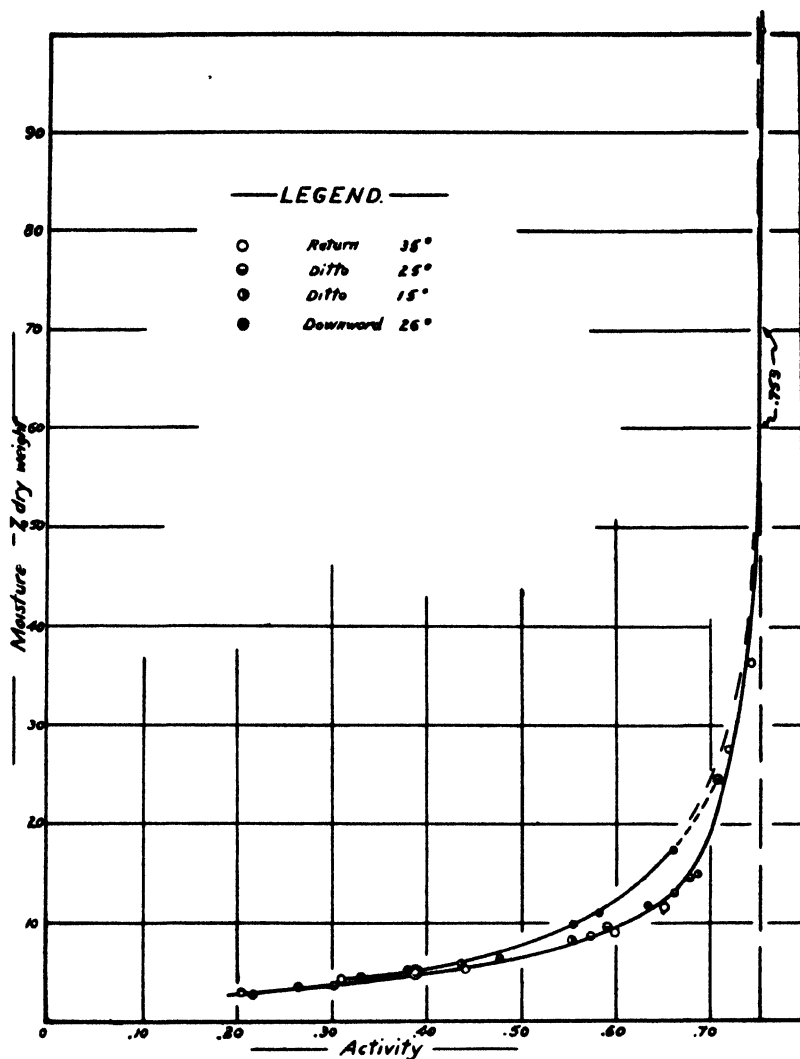
FIGURE 2. Sorption values for salt fish muscle in an atmosphere of water vapour. Upper curve indicates downward values, and lower, return values.

Brooks (1934) found the same equilibrium values for the dried weight of the sartorious of the frog determined by the two methods described by him, and Pidgeon (1934a) has shown that small traces of air make no difference in the amount of hysteresis in silica gel. In obtaining the results given above, no rigorous attempt was made to remove the last traces of permanent gases from

the samples. Hampton (private communication) has interpreted results obtained by him, using relatively large samples, to mean that differences are to be expected. His results, obtained by suspending samples in a controlled air stream, do not agree with those given above over all sections of the curve. He points out, however, that the accuracy of control of relative humidity of the ambient air was not satisfactory at high activities.

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EFFECT OF PRECOOLING AND RATE OF FREEZING ON THE QUALITY OF DRESSED POULTRY[1]

By L. Sair[2] and W. H. Cook[3]

Abstract

The rate at which poultry is frozen has been shown to have no effect on the number of bacteria present, and little, if any, effect on the extent of surface desiccation or development of visceral taint. The development of taint appears to depend primarily on the period during which the product is held at temperatures above the freezing point, and little advantage is gained by freezing promptly after slaughter, since taint development occurs during thawing.

A quantitative study of the amount of fluid exuded (drip) after freezing and thawing whole birds shows that, regardless of the rate of freezing, the whole bird does not drip. Freezing does change the condition of the water in the muscle, however, since drip can be obtained from minced meat after freezing. If minced meat is frozen within 3 hours of slaughter, the amount of drip is somewhat variable but apparently independent of the rate of freezing. If the birds are stored for 24 hours or more at 0° C., prior to freezing, the typical curved relation between the amount of drip and the freezing rate is obtained, the drip decreasing as the freezing rate increases.

Using a constant rate of freezing (2 5 hours to pass from 0° to −5° C.), the amount of drip decreases as the storage time prior to freezing is increased. During storage at 0° C., the greatest decrease occurs during the first day, but continues for periods up to 2 weeks. At 10° C., little decrease occurs during the first 5 days, after which it decreases slowly until the product spoils. The amount of drip obtained at a given rate of freezing appears to be proportional to the amount of fluid obtained from the unfrozen material, showing that the drip is determined by the condition of the water in the original minced muscle. There were some indications that the state of the water in the tissue was partly determined by the pH, but the results were not conclusive.

Introduction

This investigation was undertaken to determine the effect of the rate of freezing on the quality of dressed poultry to be stored in the frozen state. When these experiments were under way it was found that the effect of the rate of freezing was conditioned by the treatment which the product received before freezing. The scope of the study was therefore enlarged to include the effect of precooling and storage prior to freezing.

It has been shown (2, 19, 20) that when fish and beef are frozen slowly in air, they exude a certain amount of fluid or "drip" when thawed. In addition to the quantitative losses, the eating quality also suffers, presumably due to the loss of certain of the flavor constituents (19, 21). The cause of drip is

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generally attributed to the formation of large ice crystals during slow-freezing (14, 17, 20), which exert a mechanical force (4) that may rupture the cells (11, 19) or prevent the cell constituents from reabsorbing the moisture (21), either because the rate of thawing exceeds the rate of diffusion back into the cells (19), or because the proteins are denatured by the processes involved in slow-freezing (5, 12, 16). Whether drip results from only one or a combination of these possible effects is not known, but it has been found (19, 20) that rapid freezing reduces both the size of the ice crystals and the amount of drip obtained when the product is thawed.

By analogy, it would appear that all meats should drip when thawed after slow-freezing. This does not appear to be the case, since slowly frozen pork and mutton (2, 19) evidently do not drip to any extent when thawed. The claimed superiority of quick-frozen poultry (10) appears to be based on the assumption that it is affected by freezing in a manner similar to beef and fish. On the other hand, it appears to be doubtful whether slowly frozen poultry does drip (10), but no direct evidence is available. Other investigations (13, 18) indicate that slow-freezing does not affect the eating quality. Further advantages have been claimed for the quick-freezing of poultry. These include: a reduction in the number of bacteria (7); a reduced development of visceral taint (10); and superior bloom and appearance, since the rate of surface desiccation is reduced (10, 22).

Most of these claims are based on the results of practice rather than experiment, and although their validity is not questioned, it seems probable that the commercial operator interprets "quick-freezing" in terms of a whole series of processes, rather than as the strict rate of freezing which the term implies. For instance, where quick-freezing is practised, the period between slaughter and freezing may be reduced, the birds eviscerated, and the entire marketing and distributing chain improved. The over-all process may therefore result in a superior product which cannot be attributed entirely to the rapid rate of freezing.

The major part of this investigation was devoted to the question of drip in frozen poultry. The effect of the rate of freezing on bacterial count, visceral taint, and surface desiccation was investigated in a preliminary way, and the results of the tests will be reported first.

Number of Bacteria

Haines (6) has shown that the temperature of freezing between $-5°$ and $-70°$ C., *i.e.*, rate, has little effect on the ratio of viable organisms before and after freezing. On the other hand, Heitz and Swenson (7) report that the number of bacteria on slow-frozen ducks is 1000 times greater than that on the quick-frozen product. Although this difference is attributed to quick-freezing, it appears that the two lots of ducks used were not subjected to exactly the same treatment, and the observed differences may therefore be due to other factors than the rate of freezing.

In order to provide material for these tests, chickens were stored for 3 weeks at 0° C. (32° F.). Since bacterial development is greatest on the surface, the meat was removed, minced, mixed, and allowed to stand overnight at room temperature. It was then placed in small metal containers, frozen at various rates, and stored for 2 days at −40° C. (−40° F.). The samples were thawed by placing them in air at 15° C. (60° F.) for 4 hours. They were then ground with sterile sand and extracted with sterile water. Counts were made after plating appropriate dilutions on beef-extract agar and incubating for 48 hours at 25° C. A second experiment was conducted in a similar manner except that the chicken meat was transferred directly from the room at 0° C. (32° F.) to the freezing chambers, and ground in the frozen condition.

The results obtained are given in Table I, from which it is evident that the rate of freezing has little, if any, effect on the number of bacteria present. This finding appears to be in agreement with Haines (6). In practice, the quick-frozen product is usually frozen shortly after slaughter, whereas the slow-frozen product is frequently stored at a temperature near the freezing point for several days before freezing. Since bacterial growth at 32° F. appears to be relatively slow (8) it is difficult to account for the results of Heitz and Swenson (7), even on the basis of somewhat delayed freezing of the slow-frozen product. It appears that their material must have been exposed to temperatures above the freezing point for a considerable period, or else the material was not comparable in other ways. It is concluded from the results of the present investigation that quick-freezing confers no advantage over slow-freezing, from the standpoint of the bacterial numbers in properly handled poultry.

TABLE I

EFFECT OF RATE OF FREEZING ON NUMBER OF BACTERIA IN MINCED CHICKEN MEAT

Rate of freezing (0° C. to −5° C.), hr.	Bacterial count per gm. of meat, log_{10}	
	Exp. 1	Exp. 2
Check (unfrozen)	8 60	9.70
0 5	8 84	9.48
4 0	8 46	9 65
24.0	8 62	9.56

Surface Desiccation

Tests of the effect of the rate of freezing on surface desiccation were restricted to a study of the loss of moisture during freezing, and the effect of various other storage conditions will be described in another paper (1). The initial attempt to obtain quantitative information on this subject was made by determining the moisture content of the skin before and after freezing. This failed, owing to the marked variation in the moisture content of chicken skin, and the difficulty of obtaining a representative sample. The method finally adopted was to weigh the bird to an accuracy of 0.5 gm. before and after freezing. This gives the over-all shrinkage or loss during the freezing process, and possibly exaggerates the amount of moisture lost by the skin only.

Six birds, having an average weight of 6 lb., were precooled to 0° C. (32° F.).   Three of these were frozen by hanging in a room at −40° C. (−40° F.), and three by hanging in a room at −13° C. (+7° F.).   The time required for the centre of the birds to reach a temperature of 20° F. was 1.5 and 16 hours respectively.   The freezing process resulted in a loss of 3, 2, and 2 gm. for the quick-, and 4, 3, and 3 gm. for the slow-frozen birds.   This corresponds to a mean over-all loss of 0.08% and 0.12% respectively, or a difference of 0.04% between the two rates of freezing.   Even if this loss came entirely from the skin, it could hardly be expected to render the product at all independent of the storage conditions under which it is subsequently kept, since these will be shown (1) to have a marked effect on the rate of development of surface marking.   In commercial practice the birds would be frozen after packaging, and although this would reduce the rate of freezing, it would also reduce the shrinkage to less than the values reported above.

Development of Visceral Taint

Mandeville (10) states that one of the main advantages of quick-freezing is to prevent the development of off-flavors which take place after death; but experimental evidence to support this statement is lacking.   Commercially, quick-freezing is frequently practised on eviscerated poultry, and the benefits of these two distinct processes, evisceration and quick-freezing, may have become confused.   Nevertheless, it is possible that the rate of freezing may affect the development or transmission of taint from the viscera or other regions.

There are no adequate methods for measuring the amount of taint given off by a particular organ or region during freezing, or its effect on the odor or flavor of the meat after transmission.   In these tests the whole viscera were used and an attempt made to determine: the effect of prompt *versus* delayed freezing; the effect of both freezing and thawing separately and combined; and the effect of storage at 0° C. (32° F.) without freezing.   The method used was based on the "enfleurage" method (15), or the absorption of the odor by a fatty substance.   The general procedure consisted of removing the viscera, wrapping them in blotting paper supported by a cotton bag, and placing the whole in quart sealers previously coated thinly on the inner surface with Crisco.   The sealers were stoppered and treated in accordance with the requirements of a particular experiment.   Quick-freezing was accomplished by immersing the sealer in an ethylene glycol water bath at −33° C. (−28° F.), and slow-freezing by insulating the sealer with about one inch of cotton wool and placing in air at −13° C. (7° F.).   The exact time required for freezing under these conditions was not determined.   Thawing was accomplished in all cases by placing the sealers in a cabinet at 10° C. (50° F.) for two days. After thawing, the viscera were removed, and the sealer was stoppered tightly and left at room temperature for one day.   Five persons then estimated the intensity of the odor in accordance with the following score card: 0—no foreign odor distinguishable; 1—ethereal but not distasteful; 2—unpleasant;

and 3—intense and disagreeable. The values reported in Table II are the average of the five scores made in duplicate.

The summarized results given in Table II show some irregularity, which is to be expected in subjective tests of this sort, but they are reasonably consistent. It appears that tainting substances do develop in the viscera during slow-freezing (Exp. I), thawing (Exp. II), precooling prior to freezing (Exp. III), and storage in the unfrozen state (Exp. IV). The advantage of quick-freezing the warm viscera, observed in the first test, is not shown by the second. The elapsed time before freezing appears to be more important than the rate of freezing. The taint apparently develops largely during the thawing process. No evidence of free drip was obtained at any rate of freezing. These results indicate that visceral taint is entirely a question of the period during which the product is held above the freezing point. Evisceration rather than prompt or quick-freezing appears to be the obvious solution for this difficulty.

TABLE II
DEVELOPMENT OF VISCERAL TAINT

Exp. No.	Treatment of viscera	Processes involved in test	Intensity of odor* (average score of 5 persons)	
			Slow-frozen	Quick-frozen
1	Not precooled prior to freezing	Freezing, 2 days' storage at −28° C., and thawing.	2 4	0 7
2	Not precooled prior to freezing	{ Freezing only. { Thawing only.	0 6 2.0	0.4 1.4
3	Precooled 24 hr. before removal from bird	Freezing, 2 days' storage at −28° C., and thawing.	1 7	1 4
4	Not precooled prior to placing in jar	Not frozen, stored for 6 days at 0° C.	2 8	

* *Maximum intensity score −3 0.*

It is difficult to interpret the taint intensities observed in terms of flavor of the meat. Poultry stored at 0° C. (32° F.) for six days, or even longer, is generally considered of good eating quality, but this treatment resulted in the most intense odor recorded. Possibly, more taint would be developed by the viscera during storage in the frozen state, a condition not included in these studies. Since the amount of taint developed during frozen storage would be more likely to depend on the temperature and other storage conditions than on the rate of freezing, this fact would appear to furnish a further reason for evisceration.

Exudation of Tissue Fluids (Drip)
METHOD

It is convenient at this point to present the method employed for determining the amount of fluid exuded after freezing, *i.e.*, drip. Essentially, it

consisted of a slight modification of that used by other investigators (2, 17) for determining the drip in beef and fish. Other methods were also studied for purposes of comparison and will be discussed later in relation to the results obtained.

A sufficient quantity of meat for the experiment in question was obtained from several chickens, ground, and thoroughly mixed. Samples of about 100 gm. were taken, placed in tared metal dishes, about 3 in. in diameter and 1 in. deep, having removable tops and bottoms. The samples were weighed accurately, frozen at the desired rate, stored at the freezing temperature for at least 2 days, and then thawed by placing them in a tight-fitting cylinder, jacketed with water at 15° C. Thawing by this technique required about 4 hours. The drip was then determined by replacing the tops and bottoms of the cans with several layers of blotting paper, the layers being held in place by a weight equivalent to 1 gm. per sq. in., and allowing them to stand for 20 hours, at 0° C. The blotters were removed, the sample reweighed and the loss computed. The loss in weight of an unfrozen control sample was similarly determined and subtracted from that of the frozen sample to obtain the quantity reported as net drip. Usually triplicate, and frequently quadruplicate samples were used for each test and control measurement.

In order to obtain an estimate of the accuracy of this method, the standard error of the mean of triplicate samples was computed for 20 determinations chosen at random. It was found to be $\pm 0.53\%$. Similar calculations for the controls, which had a lower total loss of weight, gave an error of $\pm 0.20\%$. The standard error of the difference, *i.e.*, net drip, would therefore be $\pm 0.57\%$, and the 5% point approximately 1.1%. Differences in net drip greater than 1.1% can therefore be considered significant.

DRIP IN RELATION TO RATE OF FREEZING

To determine the amount of drip obtained at different rates of freezing, preliminary experiments were made on: eviscerated whole chickens; skinned half chickens; meat cut into approximately 1-in. cubes; minced dark meat; and minced light meat. The birds frozen whole were eviscerated to avoid the possible accumulation of drip in the body cavity, and the skin was removed from the half-birds used in the second experiment to prevent it from retaining any drip. The method employed in these instances was to place the birds in moisture-tight cans, freeze them at the desired rate, thaw at 10° C. for 2 days in a manner which permitted any liquid to accumulate in the bottom of the can, and then measure the amount of liquid. This procedure gives an estimate of the free drip plus any liquid that may have evaporated from the birds and condensed on the can during the freezing process. The cut and minced meat samples were tested by the method already described.

The results obtained are reported in Table III. It is evident that the amount of drip obtained from whole birds is negligible regardless of the rate of freezing. Removal of the skin, cutting, and mincing the meat progressively

TABLE III

EFFECT OF RATE OF FREEZING ON WHOLE BIRDS, AND CUT AND MINCED POULTRY MEAT

Material		Freezing rate: time to pass from 0° C. to −5° C. (32° F. to 21° F.)		
		1 hr.	8 hr.	18 hr.
Whole birds (eviscerated)*	Drip %	0.05	—	0.15
Half birds (skinned)*	Drip %	1.0	—	1.9
Cut meat, (1-cm. cubes approx.)†	Drip %	1.4	2.3	2.5
Dark meat (minced)†	Drip %	2.0	6 5	6.8
Light meat (minced)†	Drip %	2.9	7.4	8.7

* *Drip estimated from weight of free liquid obtained.*

† *Drip estimated from differential weight loss of frozen and unfrozen samples after absorption of liquid with blotting paper.*

increase the amount of drip obtained, and on the minced tissue the effect of different freezing rates can easily be ascertained. Although all the methods indicate that the amount of drip decreases as the freezing rate increases, the observed differences in the amount of free drip obtained from the whole, and half chickens could be attributed to experimental error. The observed differences, using cut meat and absorption with blotting paper, are too small to establish any definite relation between drip and freezing rate, so that minced meat was used for subsequent measurements. The results in Table III show that light meat yields more drip than dark meat, but as the difference was not great, both kinds were mixed and ground together in preparing material for later work.

Before initiating the main series of investigations, a number of chickens of different weight (age) from different sources were studied. All of these yielded essentially the same amount of drip at the same freezing rate. It was found, however, that the period allowed for precooling between slaughter and freezing did affect the quantity of drip obtained. In consequence, two post-slaughter treatments were used; in one, the meat was minced and placed in the freezer within 3 hours of slaughter, and in the other, the birds were pre-cooled for 24 hours at 0 °C. (32° F.) before being cut up, minced and frozen. Unless otherwise stated, the birds were all starved for 24 hours prior to slaughter.

The effect of various rates of freezing on drip formation is given in Fig. 1 for minced meat frozen within 3 hours of slaughter, and for minced and cut meat after 24 hours precooling. The rate of freezing is expressed as the time required for the product to pass from 0° C. (32° F.) to −5° C. (21° F.). As indicated by the results given in Table III, cut meat yields less drip than comparable minced samples. The curves from the cut and minced meat, however, have the same general form. After precooling the birds for 24 hours, the quantity of drip obtained from minced meat decreases with the rate of freezing. The quantity of drip increases quite rapidly up to about a 4-hour freezing period, after which it increases relatively slowly so that longer freezing

times give about the same amount of drip.   This is the typical relation which
other investigators (11, 17) have found with beef and fish.   It is evident from
the curve that a freezing time of about an hour is required if the quantity of
drip is to be reduced to half that obtained by slow-freezing.

The amount of drip obtained from minced chicken meat frozen within
three hours of slaughter was somewhat variable, but was always equal to,
or greater than, the maximum quantity obtained from birds that had hung
overnight (Fig. 1).   The fact that large quantities of drip were obtained from
quick-frozen material of this sort indicates that some post-mortem change
takes place during precooling, which tends to reduce the quantity of drip
obtained.   The relatively constant quantity of drip obtained at all freezing
rates may, therefore, be the result of two opposing tendencies, slow-freezing
tending to increase drip, but allowing the post-mortem changes which reduce
it to take place and *vice versa*.
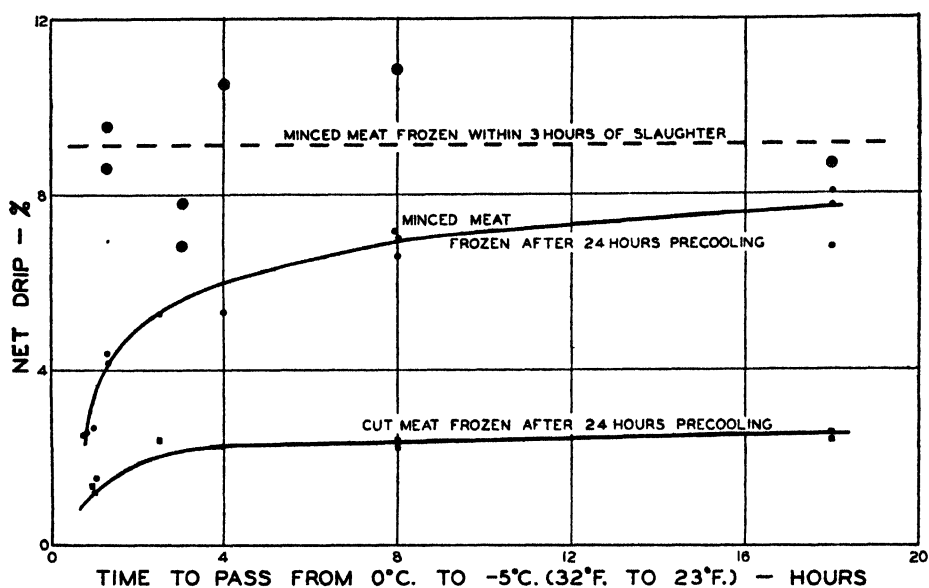
FIG. 1.   *Drip in relation to rate of freezing.*

It should be noted that although the drip from minced meat is relatively
high and is related to the rate of freezing, whole chickens show negligible
drip at any freezing rate.   This fact brings up the question as to the nature
of the deterioration, if any, that occurs when chicken meat is frozen.   In
other words, does the change in the water relations caused by freezing, as
evidenced by the drip in the minced meat experiments, cause deterioration,
or must this drip escape from the tissues before any effect is noted?   If the
latter view is accepted, then the rate of freezing has no effect on the quality
of poultry since no fluids escape from the whole carcass.

DRIP IN RELATION TO STORAGE BEFORE FREEZING

Further investigations were undertaken to determine the effect on drip of storage prior to freezing, since investigations (4) on beef indicate that the period between slaughter and commencement of freezing had no definite effect on the quantity of drip obtained. One rate of freezing was used throughout, *i.e.*, 2.5 hours to pass from 0° C. to −5° C., the product being stored at 0° C. (32° F.) and 10° C. (50° F.) for various periods prior to freezing. The higher storage temperature was used to determine whether the observed changes occurred more rapidly as the temperature increased.
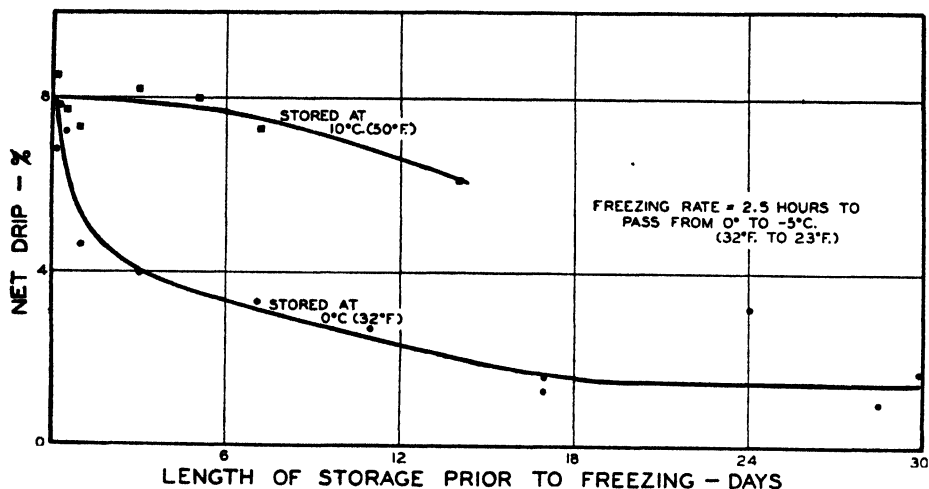
FIG. 2.   *Drip in relation to storage before freezing.*

The results obtained were used to construct the curves in Fig. 2. It is evident that storage at 0° C. prior to freezing decreases markedly the amount of drip during the first 24 hours, after which there is a slow decrease during 18 days' storage. Beyond that time the drip is more variable, probably due to incipient putrefaction, but appears to remain reasonably constant throughout the storage of the product at this temperature. This change in the quantity of drip obtained during storage cannot be attributed to evaporation since all birds were stored in closed containers at 100% relative humidity. The birds stored at 10° C. (50° F.) showed a constant drip of about 8% for the first 6 days after which it fell off slightly until the 14th day when the test was terminated owing to putrefaction of the meat.

These experiments show that certain rigor and post-rigor changes occur during storage at 0° C. which tend to reduce the drip obtained after freezing. These changes do not take place to any extent at 10° C. It is possible that two types of change can take place: one during rigor, which causes a rapid increase in the water-retaining capacity of the tissue; and another which acts in the same direction but more slowly, as indicated in the lower curve. If this explanation is correct, the difference between the curves obtained at

0° C. and 10° C. is that the first change does not occur at the higher temperature.

These results, which are typical of many more experiments, show that the amount of drip obtained depends on the initial condition of the tissues as well as on the rate of freezing. Further evidence favoring this view arose from the observation that when the drip in the unfrozen control was high, the total drip in the frozen sample at a given rate of freezing was also high, and *vice versa*. The difference between these two quantities, or the net drip, usually showed the same behavior. In order to confirm this observation, the percentage of total drip obtained from a number of frozen samples was plotted against the drip obtained from the corresponding unfrozen controls. The resulting graph is shown in Fig. 3. It is evident that the amount of drip
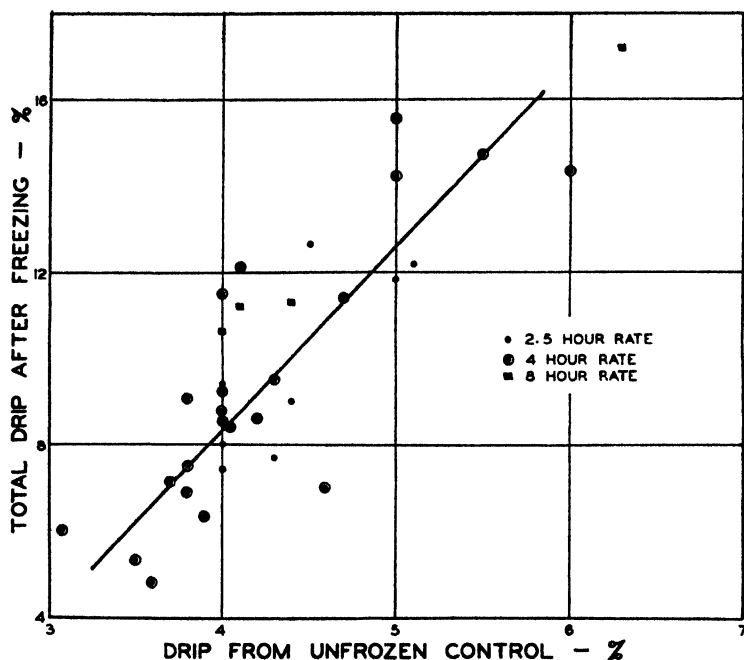
FIG. 3. *Drip in relation to condition of muscle.*

obtained after freezing depends on the tenacity with which it is held in the original unfrozen sample. Storage prior to freezing causes the tissues to retain their moisture more firmly, but other factors may also affect the condition, and the nature of the changes that take place is still obscure.

DRIP IN RELATION TO CONDITION OF MUSCLE TISSUE

In an attempt to determine the nature of the conditions governing the state of water in muscle tissue, and consequently the amount of drip, the first experiment undertaken was an analysis of the drip. The drip was obtained from approximately 500-gm. samples of meat, by subjecting the thawed

samples, contained in narrow mouthed percolators, to a pressure of 0.35 lb. per sq. in. for 3 days at 0° C. (32° F.). A slight suction was applied from time to time to remove the free liquid which collected in the voids. Samples of chicken meat were obtained immediately after slaughter, and also from birds stored 24 hours before mincing and freezing. Both tests included an unfrozen control and samples frozen at two rates. The quantity of drip thus obtained provided material for analyses and also established that there was a marked relation between the amount of free drip from minced tissue and the quantity obtained by the blotting paper method. After three days, when the last of the drip had been removed, 500 ml. of 2% KCl was added to the percolators, allowed to stand for 24 hr., and then drained off for analysis.

The results of the analysis performed on the exuded fluid and percolate from each sample are reported in Table IV. It is evident, in spite of the fact that the amount of drip obtained from the different samples varied in accordance with the treatment and freezing rate, that the composition of these fluids, with respect to the analyses performed, was essentially the same for the material frozen immediately or after 24 hours, and for the unfrozen or frozen samples. It appears, therefore, that the fluid exuded from the tissue is of relatively constant composition regardless of the treatment, or the amount obtained. This finding is in agreement with that obtained by Empey (4) on beef.

TABLE IV

COMPOSITION OF DRIP

Rate of freezing (time to to pass from 0° C. to −5° C.), hr.	Free drip, %	Original drip					Percolates	
		pH	Total solids, %	Non-combustible solids, %	Protein nitrogen, %	Non-protein nitrogen, %	Total nitrogen, %	Organic matter, %
Frozen within 3 hr. of slaughter								
Unfrozen control	5 7	6 15	14 8	1 20	1 68	0 48	0 68	5.0
1.3	14 3	6 20	15 0	1 24	1 60	0 48	0 68	4 8
18.0	14.4	6 05	14 7	1 23	1.76	0.48	0 66	5 3
Stored for 24 hr. at 0° C. prior to freezing								
Unfrozen control	4.2	6.35	14 4	1 26	1 70	0 44	0 64	5 2
1.3	8.4	6.30	14 8	1 23	1 72	0 43	0.63	4 7
18.0	12.0	6.25	14.6	1 19	1 70	0.46	0 61	5 6

Empey found that the period between slaughter and commencement of freezing has no effect on the quantity of drip from beef. Furthermore, he states that the amount of drip is minimal in muscle tissue at about pH 6.3 and that conditions more acid than this tend to increase the quantities obtained. These results appear to be conflicting, since the formation of lactic acid in the

muscle after slaughter usually results in a gradual decrease in pH which attains finally a value considerably more acid than pH 6.3. If pH is the principal factor determining the loss of fluids, then it would appear that the drip should increase for a certain period after slaughter. Chicken meat may not be comparable with beef, but the fact that the drip definitely decreased with time from slaughter, and the apparent conflict in Empey's results, rather indicate that another factor than the pH of the tissue is involved.

TABLE V

EFFECT OF STORAGE PERIOD BEFORE FREEZING, AND pH, ON DRIP

Rate of freezing—4 hr. to pass from 0° to 5° C.

Test No.	Pre-slaughter treatment	Storage period at 0° C. (32° F.) prior to freezing, hr.	pH		Drip		
			When minced	When thawed	Un-frozen control, %	Total (frozen sample), %	Net %
1	Normal: birds pre-starved	4	5.8	—	—	15.6	—
2	24 hr. before slaughter	24	6 0	—	—	9.3	—
3		144	6.1	—	—	8 4	—
4	Birds pre-starved 24 hr.	4	6.0	—	—	11.9	—
5	received injections of in-	24	5.8	—	—	5 2	—
6	sulin prior to slaughter	144	6.0	—	—	4.3	—
			(Uncut meat)				
7	Normal: birds pre-starved	0.5	7.0	6 3	6 0	14.3	8.3
8	24 hr. before slaughter	24.0	5 5	5.9	—	9 0	—
9	Birds pre-starved 24 hr.	0.5	7 2	6.6	5 0	14.4	9 4
10	received injections of sodium iodoacetate prior to slaughter	24.0	5.6	6.1	4.3	9.5	5 2
11	Normal: birds pre-starved	24	—	5.9	4.1	12 1	8 0
12	24 hr. before slaughter	120	—	5 8	3.7	7.0	3 3
13	Pre-starved for 24 hr. and	24	—	6 2	3.8	6.8	3 0
14	exercised prior to slaugh-ter	120	—	6.1	4.6	7.2	2 6
15	Fed until slaughter	14	—	5.9	3.8	9.1	5.3
16		120	—	5.9	3.9	6.4	2.5
17	Birds received injections of	24	—	5.8	4.7	11.4	6.7
18	both insulin and glucose prior to slaughter	120	—	5.7	3.8	7.5	3.7

These considerations led to some experiments in which the pH as well as the drip was studied, after various storage periods. Preliminary measurements on the pH of minced chicken muscle indicated that it usually fell within the narrow range of 5.8 to 6.1. Attempts were therefore made to extend the range of pH values obtained. Three experiments of this sort were made.

The first two were concerned primarily with the changes in pH and drip within 24 hours after slaughter, and the third with the changes that occur after a 24-hour storage period. The initial experiment included normal, pre-starved birds, and similar birds that received 120 units of insulin (3) prior to slaughter. A comparison of the changes that occur in normal, pre-starved birds, with those that take place in similar birds injected with 0.04 gm. of sodium iodoacetate (9) prior to slaughter was made in the second experiment. The final test included the following treatments: normal, pre-starved birds; birds similarly starved but vigorously exercised for 10 min. before slaughter; birds fed until slaughter; and birds that received both intravenous glucose and subcutaneous insulin 90 min. before slaughter. All measurements on this material were made 24 hours or more after death.

The results of the experiments are given in Tables V and VI. All the pH measurements were made with a glass electrode and the values are corrected to 20° C. (5), although the majority of the observations were made at lower temperatures. Owing to the limited amount of material available for the first experiment, only the total drip from the frozen sample was tested. The results show that the pH of tissue from both the normal and insulin-treated birds was quite similar at the time of mincing, and changed very little with time of storage. On the other hand, the quantity of drip from the insulin-treated birds decreased more rapidly during storage than did that from the normal birds. This suggests a relation between carbohydrate metabolism and drip, although the pH values show no evidence of any great difference in the amount of acid produced. In the second experiment, it was found that the injection of iodoacetic acid only maintained the neutral condition for a matter of 2 hours (Table VI) or less, after which it behaved as the normal sample. Therefore, no conclusion is possible from this test, since the pH and drip values were the same from both normal and treated birds.

The final experiment involved a number of treatments, and the drip and pH (when thawed) measurements were made one and five days after slaughter. In all cases, the drip decreased during storage and the tissue tended to become slightly more acid. The pre-starved and exercised birds were the most alkaline throughout and showed the least drip. This result appears to support Empey's findings. The pH and drip for the other treatments were essentially the same throughout.

Investigations relating to pH and drip formation in chicken meat are complicated by the fact that no evidence of free drip from the whole carcass was obtained. This,

TABLE VI

CHANGES IN PH OF CHICKEN MUSCLE DURING STORAGE AT 0° C.

Time from slaughter, hr.	pH of whole muscle	
	Normal birds	Birds injected with sodium iodoacetate
0 5	7.0	7 2
1 5	6 5	7 3
4.0	6 2	6 3
6 0	5.6	5.8
24.0	5.5	5.6

together with the possible variable behavior of the muscle from different chickens, led to a study of drip in relation to the effect of pH and storage on beef, pork, and mutton. The results of these investigations will be reported shortly.

It is of interest to report the observation that the pH appears to affect the color of the muscle. In these experiments, the pH range obtained in samples 24 hours after slaughter was relatively narrow, yet the more acid samples had a light pink color while the more alkaline samples had a darker appearance, tending toward a brown. This observation may be of some significance and is being studied in a more quantitative manner on other meats.

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RELATION OF pH TO DRIP FORMATION IN MEAT[1]

By L. Sair[2] and W. H. Cook[3]

Abstract

The quantity of drip obtained from meat frozen at a constant rate is affected by the period between slaughter and freezing, and the pH of the tissue. These two factors appear to act independently, and only the latter was studied extensively. In precooled meats the maximum amount of drip was obtained at about pH 5 2, and as the pH increased the net drip decreased to zero at about pH 6.4. Pork, beef and mutton behave similarly both with respect to the form of the drip-pH relation and the quantity of drip exuded at a given acidity. Beef is normally more acid than the other meats tested, and this can account for its greater tendency to drip in commercial practice.

Microscopic studies showed that large crystals were always produced by slow-freezing, regardless of the pH of the material. The absence of drip from slowly frozen tissue at pH 6 4 is therefore not due to crystal size, but must be attributed to the greater re-absorbing power of the proteins in this region. Protein denaturation does not affect the quantity of drip obtained when meat is slowly frozen or stored for periods up to three days in the freezing zone. The weak re-absorptive power of the proteins at pH 5 2 must be attributed to their isoelectric condition in this region, rather than to their denaturation. It is only in this isoelectric region that the production of small crystals by quick-freezing will reduce the quantity of drip.

Introduction

This investigation is concerned with the effect of the length of storage period between slaughter and freezing, and the pH of the tissues, on the amount of liquid exuded (drip) from beef, pork and mutton after freezing and thawing. Previous investigations on this subject have been concerned mainly with the effect of freezing and thawing rates on the exudation of drip, and little attention has been given to the condition of the muscle tissue in relation to the amount of drip obtained after freezing. During an investigation of the effect of freezing rate on the quality of poultry (11), it was found that the amount of drip obtained at a given rate of freezing decreased rapidly during the first day after slaughter, and continued to decrease at a much slower rate over a 4-week storage period at 0° C. Other evidence indicating that the condition of the tissues has some effect on the exudation of drip is obtained from the fact that pork and mutton normally do not drip to the same extent as beef when frozen at similar rates.

Several hypotheses have been offered to explain the cause of drip. Since rapid freezing reduces the size of the ice crystals, and also reduces the amount of drip exuded from such meats as beef, these two observations have been associated and the cause of drip explained in terms of various effects produced by the large ice crystals (3, 10, 12). Others (5, 9) attributed drip to both crystal size and protein denaturation, since it was found that the time and temperature conditions necessary to produce large crystals are also those which produce a rapid rate of denaturation. Most of these hypotheses fail to explain why different meats react differently to the same freezing treatment.

Empey (4) has investigated the drip from beef in relation to the period between slaughter and freezing, and the pH of the tissues, as well as the freezing rate. Contrary to the findings of most other investigators, he reports that rapid freezing does not diminish drip. He also concludes that the period between slaughter and freezing does not influence drip formation. With respect to pH, he found that although the quantity of drip did not bear a definite relation to the pH value of individual muscles, there was a zone in the vicinity of pH 6.3 in which the drip was minimal. Since the reaction of beef muscle changes from about pH 7.0 to pH 5.5 within a day or so after slaughter, his conclusion that the period between slaughter and freezing has no effect on the quantity of drip is difficult to harmonize with his conclusion that the drip is least in the region of pH 6.3.

Although Empey's conclusions do not appear to be entirely consistent, his results indicate that the pH of the tissues has some effect on the exudation of drip. In the investigation on poultry, previously cited (11), an attempt was made to relate the decrease in drip, during storage prior to freezing, to the pH changes in the tissue. The results were inconclusive. Beef, pork and mutton were therefore used in this study to determine the effect of pH on drip exudation, since these meats exhibit free drip to various degrees after similar freezing treatments.

Drip in Relation to Storage Before Freezing

Three experiments of a preliminary nature were made to determine the effect of storage, prior to freezing, on the quantity of drip obtained from beef and pork frozen at a constant rate (4 hr. to pass from 0° C. to −5° C.). The beef used for the first test was obtained from the hind quarters of a 3-year-old heifer, and that for the second test from the hind quarters of a poor quality aged cow. The pork was obtained from the hams of three pigs.

Storage temperatures of 0° C. and 10° C. were used. The meat was placed in rooms at these temperatures within one hour after slaughter. At the desired intervals, 2-lb. pieces of meat were removed, minced, placed in the freezing cans (100 gm.) and frozen. The samples were then stored at −22° C. for several days and then thawed within 4 hr. in an air bath at 15° C. Drip was determined by the blotting paper method previously described (11). Free drip was also determined on these samples after the same storage intervals by freezing approximately 1-lb. pieces, in duplicate, in closed containers and collecting the free liquid exuded on thawing.

The pH of the minced samples was taken before and after freezing, as well as after the drip determination. Twenty-four hours after slaughter, all of these observations gave similar values. The pH measurements were made with a glass electrode, and the values were corrected to 20° C., although the majority of observations were made at 0° C.

In Fig. 1, the total drip from the frozen beef and pork samples, and the drip from the corresponding unfrozen controls, have been plotted against the storage period. The number which appears beside each of the points is
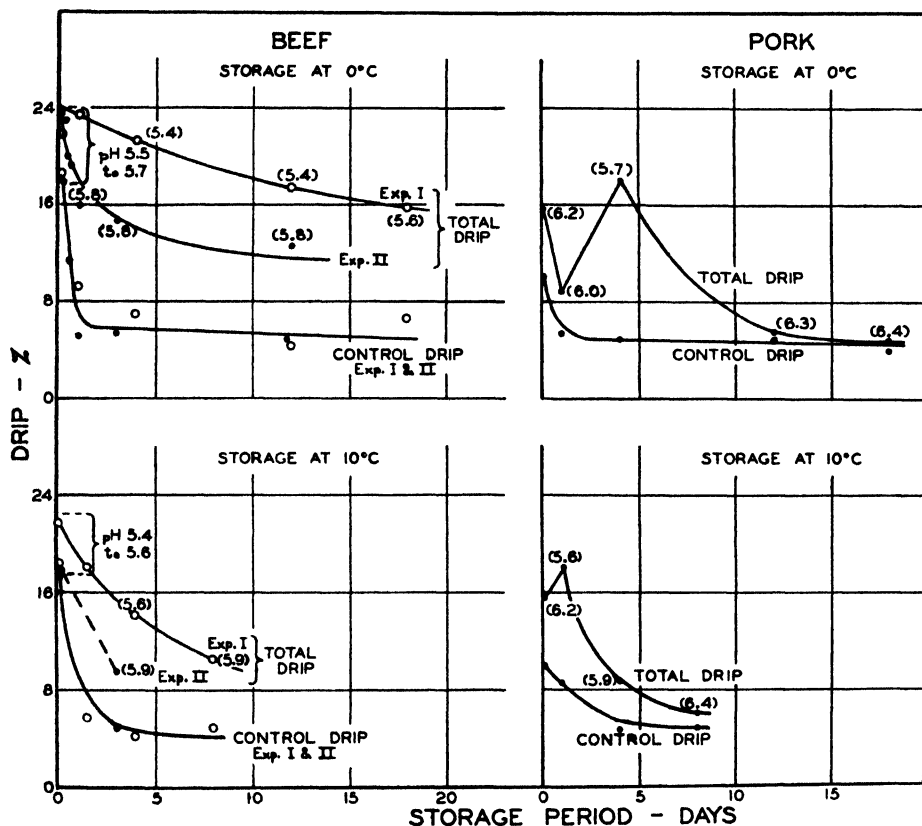
FIG. 1.   *Drip in relation to storage before freezing.*

the pH of the sample at the time of thawing. It is evident that the drip from the unfrozen (control) samples of beef decreases markedly during the first day or two after slaughter, after which it remains relatively constant. The total drip from frozen beef decreases slowly throughout the storage period at 0° C. and more rapidly at 10° C.   The pH of beef had usually fallen to a minimum value before the first drip determinations were completed, and remained relatively constant throughout the storage period at 0° C., but increased somewhat at 10° C.

The drip from the unfrozen pork samples behaved in a manner similar to beef. The total drip from the frozen samples, however, passed through a

more erratic sequence of changes during storage.   In contrast to the relatively
constant pH values observed in beef, the pH of the pork samples at first
decreased, and then increased markedly during storage.   These variations
in total drip and pH may be the result of a difference between the meat
from the three pigs used in this experiment rather than a true storage effect.

The results in Fig. 1 suggest that the quantity of drip obtained from properly
precooled material is related to the pH of the tissue at the time of freezing.
Thus the beef in the first test, having a pH of 5.4, yielded more drip after
freezing than the beef at pH 5.8, used in the second test.   The results with
pork also indicate that the maximum total drip is associated with low pH
values.

Significance of Free and Net Drip Determinations

It is appropriate to discuss here the significance of the results obtained
by the two alternative methods employed for determining drip.   Free drip
is usually determined by freezing pieces of whole muscle tissue and deter-
mining the drainage on thawing.   The net drip is obtained by determining
the amount of liquid expressed, by some adopted procedure, from both the
frozen (total) and unfrozen (control) material, the difference between these
two quantities being the net drip attributable to freezing.   In conjunction
with the experiment discussed in the previous section, an attempt was made
to determine the relation between these two quantities.   The variable nature
of the few data obtained precluded a definite conclusion, and the results
are not presented.   The observed variations can be attributed primarily to
differences in the area of cut surface, differences between different muscles (4),
and to different pH values in the whole and minced material.   Where these
factors have been controlled (7), the net and free drip appear to be in good
agreement.   The results obtained in this study, although variable, sub-
stantiated this finding for pork, and for beef frozen within 24 hr. of slaughter.
From beef stored for longer periods, the net drip obtained was about 5%
(in terms of the meat) higher than the free drip.

Since the net drip procedure employed with minced tissue gave much more
reproducible results than the free drip determination, it was adopted for
subsequent experiments.   It is doubtful if the results obtained by this method
provide a quantitative estimate of the free drip in meats subjected to different
storage and freezing treatments.   It has, however, the added advantage of
showing the effect of a given treatment on the moisture-retaining capacity
of both the unfrozen and frozen tissue.

In Fig. 1 the drip from both the frozen and unfrozen tissues has been
plotted against the storage period instead of the net drip.   It is evident that
some change occurs in the unfrozen tissue immediately after slaughter, and
that this markedly decreases the quantity of water that can be removed as
drip.   As the drip from the frozen material decreased comparatively slowly
throughout the entire prefreezing storage period, it would appear that these
rigor or post-rigor, changes affect the moisture retention of the unfrozen

material only.   If this is so, then the practice of computing the net drip by difference is unsatisfactory for meats frozen within 24 hr. after slaughter, since it combines the effect of these changes in the unfrozen sample with the effect of the freezing treatment on the frozen sample.

It appeared from these preliminary experiments that the factors determining the water-retaining ability of both unfrozen and frozen muscle tissue merit investigation.   Since the method employed for determining drip was inadequate for a critical study of changes that occur within 24 hr. after slaughter, this phase was abandoned in favor of investigating the factors that affect the quantity of drip obtained when aged material is frozen.

Relation Between Drip and pH

Since the preliminary experiments indicated that the amount of drip (from material that had been properly precooled before freezing) was dependent on the pH of the tissues, this subject was the next to be investigated.   The results of the experiments described above and those of other investigators (2, 6), indicate that the pH of beef, a few days after slaughter, is relatively constant, whereas the pH of pork is subject to some variability.   For this reason pork was used.

The meat was obtained from carcasses that had been cooled or stored for at least 48 hr. after slaughter.   Such material varied in pH from a minimum of 5.6 to a maximum of 6.3, but the majority of the samples fell within a much narrower pH range.

In order to extend this range, and also to obtain a more uniform distribution of samples within it, an attempt was made to adjust the acidity by artificial means.   The method employed was to inject the estimated amount of 85% lactic acid or 26% ammonium hydroxide into 800- to 900-gm. pieces of pork. In all cases the acid or alkali was diluted to a final volume of 20 ml. before being injected at numerous uniformly distributed points with a calibrated 5-ml. hypodermic syringe.   In producing a range of pH values by this method, some of the samples did not require adjustment and were injected with 20 ml. of water, to render them comparable with the treated material.   Following injection, the samples were stored for 3 days at 0° C. to allow the acid, or alkali, to diffuse.   They were then minced, triplicate samples removed, frozen at a constant rate (*i.e.*, 4 hr. to pass from 0° C. to −5° C.) and the same number kept as unfrozen controls.   The drip determinations on these samples were then made in the usual way.

Several objections may be raised to this artificial method of preparing the samples.   The added water, although small in proportion to the amount present in the muscle, is undesirable.   Again, the variable amount of acid or alkali present will cause some variation in the osmotic pressure.   Other objections could be detailed, but they could scarcely introduce sufficient bias to be detectable by the method employed for determining drip.   This contention was supported by the results obtained.   Reference to Fig. 2 shows

that the curves relating drip and pH in normal and treated samples coincide, within experimental error, over the pH range encountered in normal samples.

This method of adjusting the pH was then applied to beef, which normally has a comparatively constant pH that shows little change during storage (Fig. 1). A single series of experiments was also made with mutton. Casual observations made during the course of these experiments indicate that mutton behaves like pork, in that the pH is subject to considerable variation between animals, and probably increases more rapidly during storage.

The observed drip from the frozen (total) and unfrozen (control) samples and the difference between them (net drip) have been plotted against the pH in Fig. 2. It is evident from the curves that these three quantities are related in meats stored two days or more after slaughter. A pH condition which causes an increase in the drip from the unfrozen control also causes an increase in the total and net drip. This relation has previously been reported for poultry meat (11).
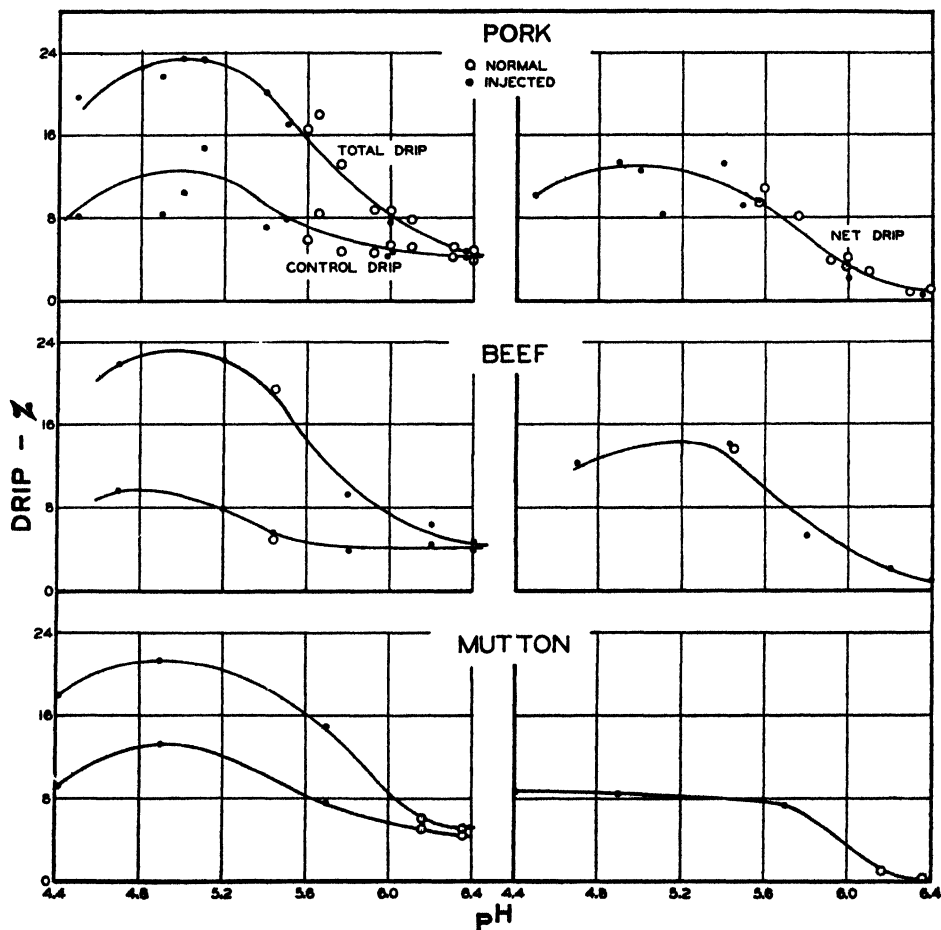
FIG. 2. *Drip in relation to pH of muscle tissue.*

The curves in Fig. 2 show a marked relation between the amount of drip and the pH; all three meats showing maximum drip between pH 5.0 and 5.2, the curves descending slightly to pH 4.4, the lowest value tested. On the alkaline side of pH 5.2, the drip decreases markedly to about pH 6.4, where the effect of freezing cannot be detected, *i.e.*, net drip is zero. A few samples having pH values between 7.0 and 9.0 were tested, and there was no evidence that the drip increased within this region.

Although the shape of the total drip-pH curves for all three meats is identical, within experimental error, the quantity of drip obtained from the unfrozen samples of beef was somewhat less than that obtained from similar samples of pork and mutton. The result is reflected in a somewhat higher net drip for beef, which is of questionable significance, since it was mentioned in an earlier section that the method used for determining net drip probably over-estimates the free drip from beef by about 5% in stored material such as that used in these tests. It is therefore concluded that these three meats will drip to the same extent after freezing and thawing, provided that they have the same pH and that the same freezing rate is used. The experience of practical operators and the results of investigation (3, 12), show that more drip is obtained on thawing slowly-frozen beef, than on thawing slowly-frozen pork or mutton. This behavior can be attributed to the pH values typical of these meats. The pH of beef is relatively constant and close to the value at which maximum drip occurs, whereas pork and mutton vary in pH from carcass to carcass, are generally more alkaline, and tend to become more so during storage. This fact can readily explain the small amount, or practical absence, of drip from frozen pork or mutton.

Relation Between Crystal Size and pH

The exudation of drip from meat after freezing and thawing is frequently attributed to various effects produced by the formation of large ice crystals during slow-freezing. As shown in the previous section, meat at pH 6.4 does not drip even after slow-freezing. In order to fit this fact into the hypothesis that crystal size determines the formation of drip, it is necessary to assume that pH affects the size of crystal formed. This assumption is not untenable, since the necessary condition for the formation of large ice crystals is that the rate of movement of water from the tissue to the existing ice nuclei during freezing exceed the rate of crystal formation as determined by the cooling rate. Since the acidity of the tissue may affect the rate of moisture movement, the effect of pH on the crystal size at various rates of freezing was investigated.

Several samples of pork and beef were adjusted to pH values varying from 5.5 to 6.5 by the procedure already described. These samples were passed through the freezing zone (0° to −5° C.) in one and in 24 hr. to produce quick- and slow-frozen material respectively. The samples were then examined microscopically with both reflected and transmitted light using material in the frozen and fixed (7) condition. Numerous examinations showed that the crystal size depended entirely on the rate of freezing, any slight effect of

pH being indistinguishable from the variations between sections of the same material. This is illustrated in Plate I, which shows typical photographs of fixed material (approx. 5×), quick- and slow-frozen at pH 5.5 and 6.5. It is evident from these results that crystal size alone is not an adequate hypothesis for explaining the formation of drip.

Effect of Freezing and Storage at Different pH Levels on Protein Solubility

Since the foregoing experiments indicate that drip is markedly influenced by the pH of the tissues, while crystal size and the structural differences between the three meats tested are of secondary importance, it would appear that the condition of the proteins must be the primary factor responsible for the retention of fluids by muscle tissue after freezing.

The fact that the maximum drip occurred in the region of pH 5.2 could be attributed to either the isoelectric condition of the proteins or to their more rapid denaturation in this region. Bate Smith (1) has shown that myosin and globulin X constitute 89% of the muscle proteins, and that their isoelectric points are pH 5.5 and 5.2 respectively. In support of the second possibility, Finn (5) has shown that denaturation (as indicated by the amount of protein precipitated from muscle juice during 24 days' storage at −2° to −3° C.) occurs readily on the acid side of pH 6.0. Certain investigators (8, 9) consider that denaturation is at least partly responsible for the exudation of fluids after freezing, storage, and thawing. Since the isoelectric point is generally the region of minimum colloidal stability, it would appear that loss of solubility, or water-retaining capacity at this point, does not necessarily indicate denaturation, or an irreversible alteration of the protein. Unfortunately this is difficult to prove since denaturation cannot be defined except in relation to the method used for its detection.

In the first experiments an attempt was made to relate the quantity of drip to the amount of nitrogen extracted by 1.2 molar potassium chloride, buffered at pH 7.2 as recommended by Reay (10). Meat samples at different pH levels were frozen at a constant rate (4 hr. to pass from 0° C. to −5° C.) and stored at −22° C. for several weeks. Another experiment, using a different sample of pork, was performed by storing the material at −3° C. for 12 days, since the rate of denaturation is reported (5, 8, 9) to be most rapid in this region.

The results appear in Table I. The pH values marked by an asterisk were obtained after the injection of ammonium hydroxide. The quantity added was equivalent to about 0.3% in terms of the extractable nitrogen, and this amount was therefore subtracted in order to obtain the reported values. The other samples had attained the reported pH values naturally. It is obvious that, although the pH and the quantity of drip varied markedly, the quantity of nitrogen extracted remained relatively constant. Similarly the amount of nitrogen extracted from the samples stored at −3° C. was quite constant and hence showed no evidence of denaturation. On the other hand

PLATE I
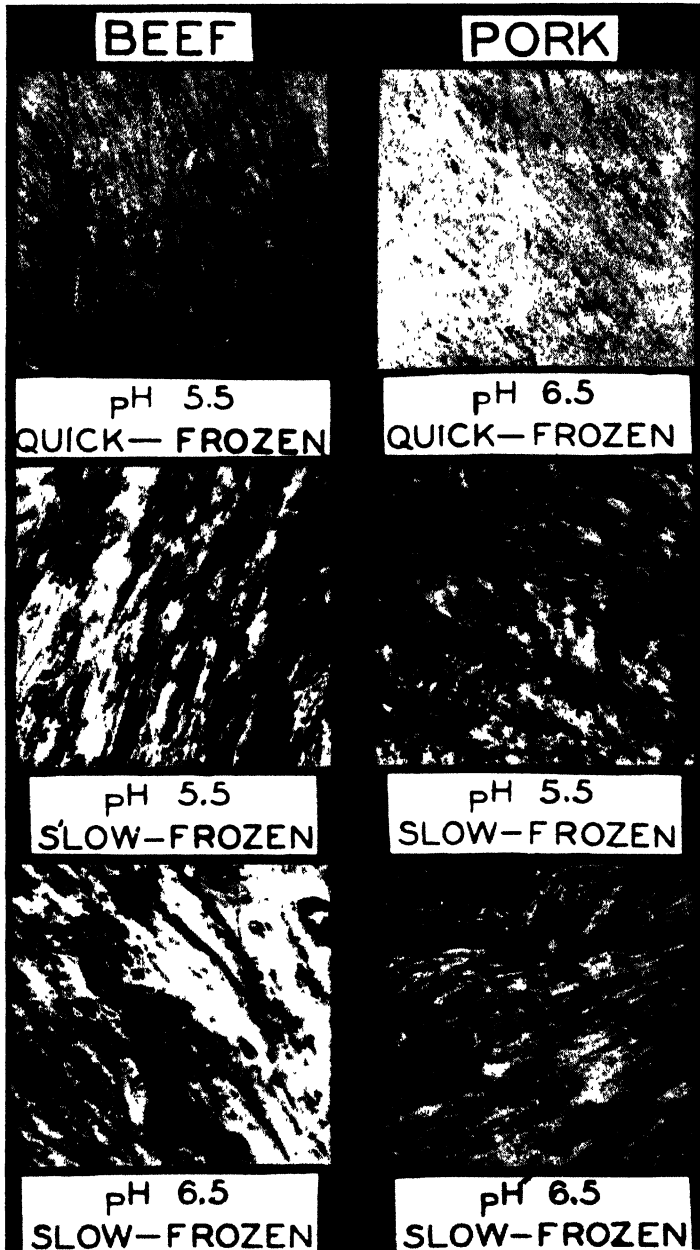
Effect of pH and freezing rate on crystal size. 5×.

the quantity of drip obtained from the stored sample did increase somewhat over that obtained from the corresponding sample representative of freezing alone. This suggests that some change occurred during storage at −3° C. but that the extraction technique did not detect it.

TABLE I

EFFECT OF pH, FREEZING, AND STORAGE ON PROTEIN SOLUBILITY

Treatment	Meat	pH	Total drip, %	Extracted nitrogen, %
Freezing only (stored at −22° C.)	Beef	5.2	22 2	1.20
	Beef	6.4*	4 8	1.14
	Pork	5.4	20.4	1.18
	Pork	6.0	7.0	1.24
	Mutton	5.7	14.9	1.26
	Mutton	6.2	6.0	1.22
Freezing only (stored at− 22° C.)	Pork	5.5	21.9	1.06
	Pork	6.2*	8.5	1.04
Freezing + 12 days storage at −3° C.	Pork	5.5	28.2	1.08
	Pork	6.2*	9.3	1.05

* Ammonium hydroxide injections necessary to attain these levels.

It would appear that an extraction procedure is not suited to a study of the nature of the changes in the proteins affecting drip. In the first place, only a fraction of the total protein is extracted, and any irreversible change that occurs at different pH values might take place in the unextracted portion. Secondly, such a method could scarcely yield an estimate of the effect of pH on a reversible colloidal condition, since the amount extracted would be determined by the pH of the extraction medium rather than by the pH at which freezing and thawing took place.

The use of expressed muscle juice for a study of the effect of freezing on the proteins also appears unsuitable. The several muscle proteins are not present in nearly the same proportions in the expressed juice (5), and the pH would require adjustment after freezing and thawing, in order to distinguish between colloidal instability at the isoelectric point and denaturation.

It is concluded from these experiments that the large difference in the quantity of drip obtained at the two pH levels used cannot be explained in terms of denaturation as evidenced by the constant amount of protein nitrogen extracted from all samples. However, since an extraction procedure appears to be unsuitable for a study of the nature of the pH-drip relation, further experiments of this sort were abandoned.

Contribution of Isoelectric Condition and Denaturation to Drip

Since methods based on the solubility of the proteins appear to be unsuitable for studying the effect of pH on drip, an attempt was made to use methods

based on the reversibility of the pH-drip relation in muscle tissue. The loss of moisture-retaining capacity at pH 5.2 may be attributed to either the iso-electric condition of the proteins (1) or to their denaturation in this acid condition (5). If the first of these factors is the cause of drip, the moisture-retaining capacity should be restored by changing the pH. On the other hand, if denaturation is the cause of drip, it might reasonably be expected to be irreversible when the pH is altered over a limited range.

The experiments undertaken included a study of the effect of freezing and storage at $-3°$ C. for various intervals. This storage temperature was chosen for reasons already given. A large sample of pork was adjusted to pH 5.4–5.5 by the injection technique already described. After standing two days, the meat was minced, mixed, transferred to the freezing cans, and frozen at the usual rate ($0°$ C. to $-5°$ C. in 4 hr.). Four series of 12 samples were prepared in this way. One series was stored at $-22°$ C., and the remaining three were held at $-3°$ C. for 3, 6, and 12 days, respectively, before transferring them to a temperature of $-22°$ C. until required.

After thawing, one set of triplicate samples from each series was kept at the original pH, and the pH of the other three sets was adjusted to pH 5.7, 6.0 and 6.4, as nearly as possible, by the addition of small increments of ammonium hydroxide. Each sample was then well mixed, stored at $0°$ C. for 4 days to allow the alkali to diffuse, refrozen, thawed, and the quantity of drip determined.

A similar piece of meat at pH 6.2–6.3 was minced, frozen, stored and treated as described above, except that lactic acid was used to obtain the required pH values.

The results obtained appear as two charts in Fig. 3. The one on the left was constructed from the data obtained from the samples that were frozen and stored at pH 5.4–5.5, and the one on the right from the data obtained from samples stored at pH 6.2–6.3. In both charts the total drip is plotted against the pH after the final adjustment, the value at which the drip deter-minations were made.

The lower heavy line in both charts is a reproduction of the curve relating total drip and pH, taken from Fig. 2. It is evident that the samples which were not stored at $-3°$ C., and those stored at this temperature for only 3 days, fit this curve within experimental error. All of the samples stored for 6 or 12 days at $-3°$ C., at either pH 5.3–5.4 or pH 6.2–6.3, yielded more drip than the material stored for shorter periods. The broken line in the two charts (Fig. 3) has been drawn through the points representing the data obtained with this material.

It appears, therefore, that the high total drip observed throughout at pH 5.2–5.4 can be completely reversed by adjusting the pH as long as the storage period in the zone of crystal formation does not exceed 3 days. Similarly, the samples stored at pH 6.2–6.3, which do not yield any net drip, showed the same amount of drip as the samples stored initially at 5.4–5.5, when adjusted to the same pH values, if stored for less than 3 days at $-3°$ C.

Since the effect of pH on drip is reversible under these conditions, it is concluded that the high drip observed at pH 5.2–5.4 is due to the isoelectric condition of the principal muscle proteins, and not to denaturation.

Although no evidence of denaturation was indicated by the drip determination up to 3 days' storage at −3° C., it is evident from the broken line that the pH-drip curve was displaced upwards in material stored for 6 or 12 days at −3° C. This increase in drip indicates that denaturation did occur over these longer storage periods, although the slope of the curve still indicates a certain degree of reversibility. Since the points obtained after 6 and 12 days' storage fall on the same curve, within experimental error, it
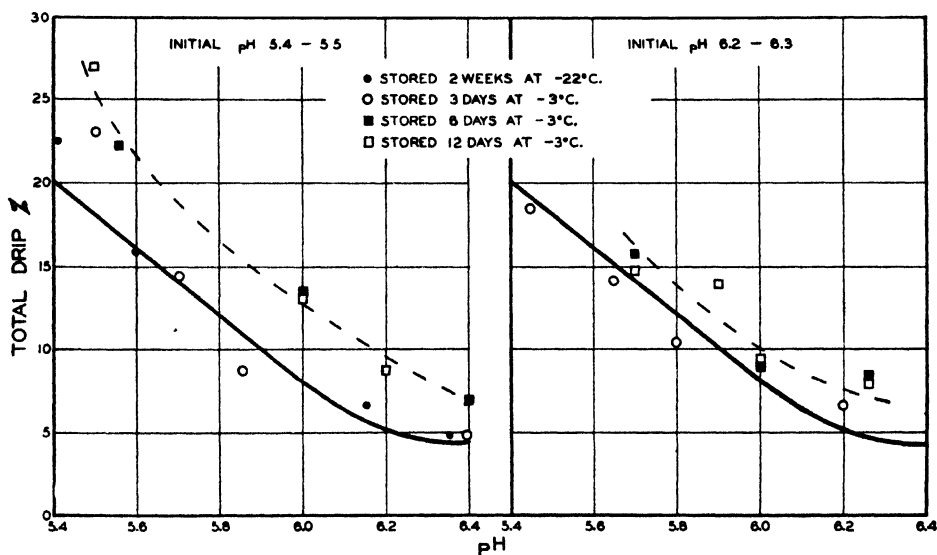
FIG. 3. *Reversibility of pH—drip relation in muscle tissue. Solid line, unstored material; broken line, material stored for 6 or 12 days at −3° C.*

appears that denaturation, as it affects drip, is complete in about 6 days. The displacement of the drip-pH curve from that of unstored material is less at pH 6.2–6.3 than at pH 5.4–5.5, indicating that denaturation is more rapid or affects more of the protein in the more acid material. This confirms Finn's results (5).

Since even slowly frozen material would generally pass through the freezing zone in less than 3 days, it appears that protein denaturation is not the factor causing drip under ordinary conditions. Moreover, although the extent of denaturation is greater at pH 5.2–5.4 than at pH 6.3, this difference is small compared with the direct effect of pH on drip. It is therefore concluded that the observed dependence of drip on pH is due primarily to the isoelectric condition of the muscle proteins rather than to their denaturation.

Relation of pH to Change of Weight in Salt Solutions

Further evidence indicating that a portion of the water present in muscle tissue is held less firmly at pH 5.5 than at higher pH values was obtained by

observing the changes in weight of pork placed in dry salt or saturated salt solutions. These preliminary experiments were made with 100-gm. pieces of pork, varying in pH from 5.0 to 6.4, by immersing them in saturated sodium chloride solution, or covering them with dry salt for periods up to 10 days, in a room at 5° C.

Reference to the results given in Table II shows that the change in weight is markedly influenced by pH. The samples having a pH of 5.5 underwent no appreciable change in weight in the salt solution, but lost 32.9% of their weight in the dry salt. Samples having a pH of 6.4 gained 15.3% in weight in the salt solution, and lost only 18.6% of their weight in the dry salt. It is evident that pork tends to lose more moisture at pH 5.5 than at 6.4, a result in agreement with the observations made on drip.

TABLE II

EFFECT OF pH ON WEIGHT AND SALT UPTAKE OF PORK DURING PICKLING

Pickling method	pH of meat	Change in weight in			Salt content of pickled meat, %
		5 hr., %	24 hr., %	10 days, %	
Saturated salt solution	5 0	−3.2	−2.9	− 2 7	17.3
	5.5	+0.2	+0.9	+ 0 1	17.2
	6.0	−0.7	+0.8	+ 0.8	17.4
	6.4	+2.8	+6.3	+15.3	17.0
Dry salt	5.0	—	—	−34.0	15.8
	5.5	—	—	−32 9	16 3
	6.0	—	—	−19.2	16.0
	6.4	—	—	−18.6	17.2

The above results are preliminary to a more extensive investigation dealing with the effect of pH on salt penetration. Callow (2) has found that salt penetrates acid samples (pH 5.5) more rapidly, a finding that may also be related to the isoelectric condition of the proteins.

Relation of pH to the Color of Meats

In the last investigation it was noted that the color of the salt solutions was influenced by the pH of the pork. The samples at pH 5.0 yielded a colorless solution, while the alkaline samples (pH 6.4) yielded a deep red solution. Intermediate pH values showed a gradation in color between these two extremes.

It was also observed that the color of meat itself was markedly affected by pH. As indicated previously, an injection method was used to obtain the desired pH values, and it was found that at pH 5.0 the samples became a grayish color, while those at pH 6.5 were a deep red. Intermediate pH values yielded a gradation between these extremes. On readjusting the pH of the acid samples (5.0) to a more alkaline state, the color of the meat changed from gray to red. Undoubtedly some relation exists between pH

and meat color. This was evident for pork, beef and mutton, and this relation is now being studied quantitatively in these laboratories.

Discussion

The effect of storage, between the times of slaughter and freezing, on the amount of fluid exuded by meat after freezing, could not be determined precisely by the methods employed. It appears, however, that two distinct phenomena are involved. The drip obtained from meat frozen at a constant rate is determined primarily by the amount of acid contained in the tissues, and it increases rapidly as the pH falls below 6.0. In unfrozen material the amount of fluid exuded decreases rapidly during the first day or two after slaughter, regardless of the pH changes. During this period, the difference between the amounts of fluid exuded by frozen and unfrozen material cannot be attributed entirely to the effect of freezing.

After several days' storage, the quantity of drip obtained from both frozen and unfrozen meats appears to be determined by the same factors, inasmuch as an increased drip from unfrozen material is reflected in a similar behavior of the frozen material. Meat at pH 6.4 or higher does not drip as a result of freezing at any rate requiring less than 3 days to pass from $0°$ C. to $-5°$ C. At pH 5.2–5.5 the amount of drip reaches a maximum, and in this region an increased freezing rate reduces the amount of drip obtained.

This behavior can be explained by a high water-retaining capacity of the tissue proteins at pH 6.4, resulting in the complete retention of the water produced on thawing, regardless of the size of ice crystal formed during freezing. At pH 5.2–5.5, the water-retaining power of the proteins is lower and moisture losses occur. These losses can be reduced by rapid freezing which produces smaller ice crystals, and a more uniform distribution of water when they melt. Under these conditions less fluid is exuded, although moisture can be removed more easily from tissues at these pH values, even if it is not frozen. The reduced moisture-retaining capacity of the tissue proteins at pH 5.2–5.5 appears to be attributable to their isoelectric condition rather than to accelerated denaturation in this region.

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PRECOOLING OF POULTRY

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The preparation of market poultry involves bleeding, plucking, and cooling the product to a temperature near the freezing point, before the birds are graded and packed for subsequent storage or transport. For obvious reasons this initial cooling, commonly termed precooling, should be accomplished quickly. The present study was undertaken to provide quantitative information on the time required for precooling. Other factors, such as shrinkage; retention of bloom during subsequent storage in the frozen state; and bacterial content of the water used for precooling, where this method was employed, were also investigated in a preliminary way. The effect of various cooling and freezing practices on other qualities of the product have been discussed in an earlier paper by Sair and Cook (1938).

The time required to cool the product to a given temperature depends on the properties of both the cooling medium and the product. The properties of the cooling medium—whether liquid or gas, its temperature, rate of circulation, etc.—need not be considered here, since only a limited range of these conditions is available or possible for precooling purposes and these are comparatively well understood. Any property of the product affecting its heat capacity or conductivity will also affect the precooling time. Such factors as the initial temperature and weight of bird can be estimated quantitatively and related to the time required for cooling. Other factors, such as the shape of the bird, which affects the surface-to-volume ratio; and the content and distribution of moisture and fat in the carcass, discussed by Stiles (1922), will also affect the heat capacity and conductivity, and such quantities cannot be measured with sufficient precision to determine their influence on the rate of cooling. By measuring the initial temperature, weight, and precooling time of a sufficient number of birds chosen at random, it was possible, by statistical analyses, not only to relate these three observed quantities but also to gain some idea of the relative influence of the unknown quantities from the residual variance.

PRECOOLING TIME

The birds were killed, dry rough-plucked, wax-dressed, and removed to the precooling room at once. All temperatures were taken immediately after weighing and at one-hour intervals thereafter by means of thermocouples connected to a recording instrument. In certain preliminary experiments on cooling rates in air, two thermocouples were used for each bird. One of these was placed just under the skin on the breast to determine the surface temperature and the other in the center of the bird to determine the internal temperature. In the main series of tests the internal temperature only was measured.

The air temperature in the precooling room was 0°C.(32°F.) throughout. The relative humidity was about 70 per cent initially and increased to about 75 per cent for a short time after the birds were placed in the space and then returned to the initial value. An intermittent air circulation, under the control of a thermostat, at a mean rate equivalent to about ten changes per hour was used throughout. These conditions are probably somewhat drier than those used in commercial practice.

The birds cooled in water were immersed in tanks placed in the precooling room. The amount of water used was always in excess of ten times the weight of product immersed at any one time. Under these conditions the water temperatures changed slowly and enabled the thermostatic devices to maintain the required temperatures of 0 and 7.2°C.(32 and 45°F.). The birds were placed in the tanks as soon as received and removed two hours later regardless of their internal temperature. They were then allowed to hang in the room at 32°F. to dry and finish cooling. Hourly temperature records were taken throughout the cooling period whether the birds were in water or air; and all computations were based on the time required to reach the desired internal product temperature, whether it was reached in the water or subsequently while hanging in air.

As the final stage of cooling was accomplished in a medium at 32°F. in all experiments, an internal temperature of 1.1°C.(34°F.), i.e., two degrees (Fahrenheit) higher, was taken as the end point. The time required for cooling was taken from the time the product was received at the cooling room until it reached 34°F., and was subsequently read from the chart on the recorder, interpolations being made where necessary.

The results of preliminary experiments, in which the weight and other factors were standardized as far as possible, showed that a linear relation existed between the logarithm of the product temperature and the time in the precooling room. Since the rate of

cooling is determined by the temperature gradient between the product and the cooling medium, and since air temperatures either above or below 32°F. may be used in practice, it was felt that the results would be more generally applicable if the temperature gradient rather than the product temperature was used in subsequent computations.

Typical curves obtained from the results of preliminary experiments (Fig. 1) show that the temperature gradients between the center of the product and the air; the skin and the air; and the
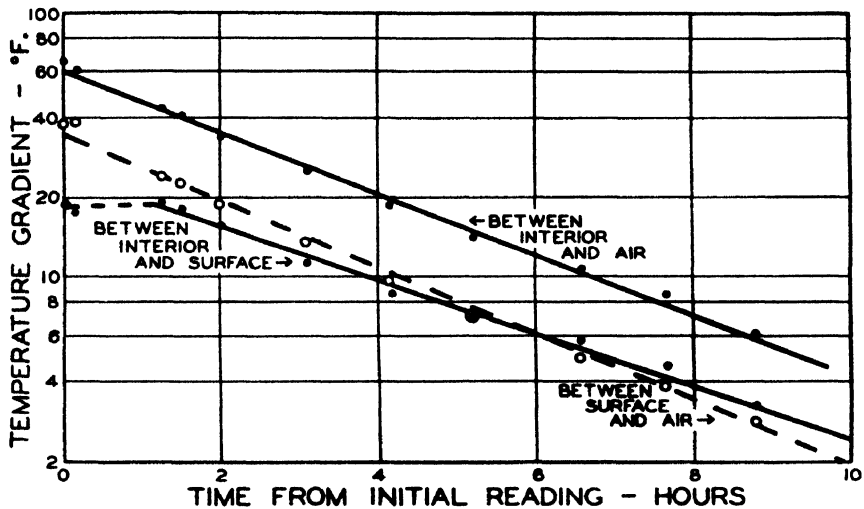
FIG. 1. Relation between logarithm of temperature gradients and time.

center and the skin, once a steady condition has been attained, are all linear functions of time within the variability ordinarily encountered. The temperature gradient between the interior of the product and the air is naturally the largest, being the sum of the other two gradients measured. It is evident that the gradient between the surface of the product and the air falls off more rapidly with time than the gradient between the product interior and the air. The reverse is naturally true of the gradient between the interior and surface of the product. The two lower curves (Fig. 1) cross six hours after the experiment was started, or generally speaking, the two gradients become equal at about half the total time required for cooling. The behavior of these two curves indicates that transfer of heat from the surface of the product to the air determines the rate of cooling during the first half of the period, while transfer of heat from the interior to the surface determines the rate during the second half. Under these conditions it would appear

TABLE 1

Regression Equations Relating the Cooling Time of Poultry to Initial Temperature and Weight

Description	Degrees freedom	Equation	No.
Cooled in air at 0°C.(32°F.)—			
Linear. Type equation [1]	...	$T = K + b_1 \log (t_p - t_m) + b_2 \text{ Wt.}$	(1)
First experiment only..........	27	$T = -7.7 + 6.84 \log (t_p - t_m) + 0.50 \text{ Wt.}$	
First and second experiments..........	56	$T = -5.0 + 6.23 \log (t_p - t_m) + 1.16 \text{ Wt.}$	(2)
Second degree. Type equation..........	...	$T = K + b_1 \log (t_p - t_m) + b_2 \text{ Wt.} + b_3 \text{ Wt.}^2$	
First experiment only..........	26	$T = -4.7 + 6.61 \log (t_p - t_m) - 1.36 \text{ Wt.} + 2.76 \text{ Wt.}^2$	(3)
First and second experiments..........	55	$T = +2.8 + 6.12 \log (t_p - t_m) + 1.23 \text{ Wt.} - 0.09 \text{ Wt.}^2$	(4)
Cooled in water at 32°F. for 2 hours then in air at 32°F.—			
Linear..........	8	$T = -0.53 + 1.26 \log (t_p - t_m) + 0.38 \text{ Wt.}$	(5)

[1] T = Time in hours to pass from initial temperature to temperature of cooling medium + 2°F., i.e., 34°F. in these tests. K = Initial constant. b_1 = Regression coefficient, temperature gradient. t_p = Initial product temperature. t_m = Temperature of cooling medium. b_2 = Regression coefficient, weight. b_3 = Regression coefficient, (weight).² Wt. = Weight in pounds.

that immersion in water for a short period after dressing would be of material assistance in reducing the time required for cooling.

In the main body of experiments it appeared preferable to omit measurements of the surface temperature of the product in favor of making more measurements on the internal temperatures of the birds. With the equipment available this enabled twice as many birds to be studied at a given time. Only one thermocouple was placed in each bird but an estimate of the standard error of the temperature gradient (i.e., a difference) indicated that it was less than one degree Fahrenheit.

In analyzing results the logarithm of difference between the initial product and room air temperatures was computed. Since this quantity is a linear function of the cooling time (Fig. 1), it was only necessary to compute a single coefficient for temperature in setting up the equations. It was not known whether the cooling time would be a first or higher degree function of the weight. Theoretical considerations indicated that it would not be linear, but in view of the relatively high variability observed, the best fit could only be determined analytically.

The partial regression equations computed from the results are given (Table 1). Precooling in air was studied most extensively and two linear and second-degree equations were fitted to the data obtained by this method. Fewer birds were used in the experiments involving immersion in water, and some of the results had to be discarded owing to the suspected entrance of water into the body cavity. In consequence, only a limited amount of curve fitting seemed to be justified. The first experiment on precooling in air included a group of birds having a mean weight of 3.5 pounds rather uniformly distributed over the weight range from one to six pounds, as shown by the black portion of the inserted chart (Fig. 2). Birds of comparable size and weight distribution were used in both experiments involving immersion in water. The first experiment on air cooling is therefore directly comparable with those in which immersion was employed. Later, another experiment was conducted on precooling in air using heavier birds (Fig. 2, hatched portion) and the results of this study were combined with those of the first in obtaining Equations 2 and 4.

From the results of an analysis of variance to test the significance of the several equations (Table 2) it is evident that the regressions on temperature difference (initial product temperature) and body weight are highly significant when compared with the residual variance. The variance due to regression on weight squared (second-degree equations) is significantly greater than the residual variance

in the first experiment only. Since the variance accounted for by this term, although significant, was relatively small, and since the second-degree term contributed nothing in the combined experiments, it was concluded that a linear equation in terms of weight gives a satisfactory fit, considering the magnitude of the residual variance.

Equation 2, derived from the majority of the experimental data, was therefore used to construct Fig. 2, which shows the time required to cool birds of various weights over the temperature intervals likely to be met in practice. The temperature axis shows the difference in
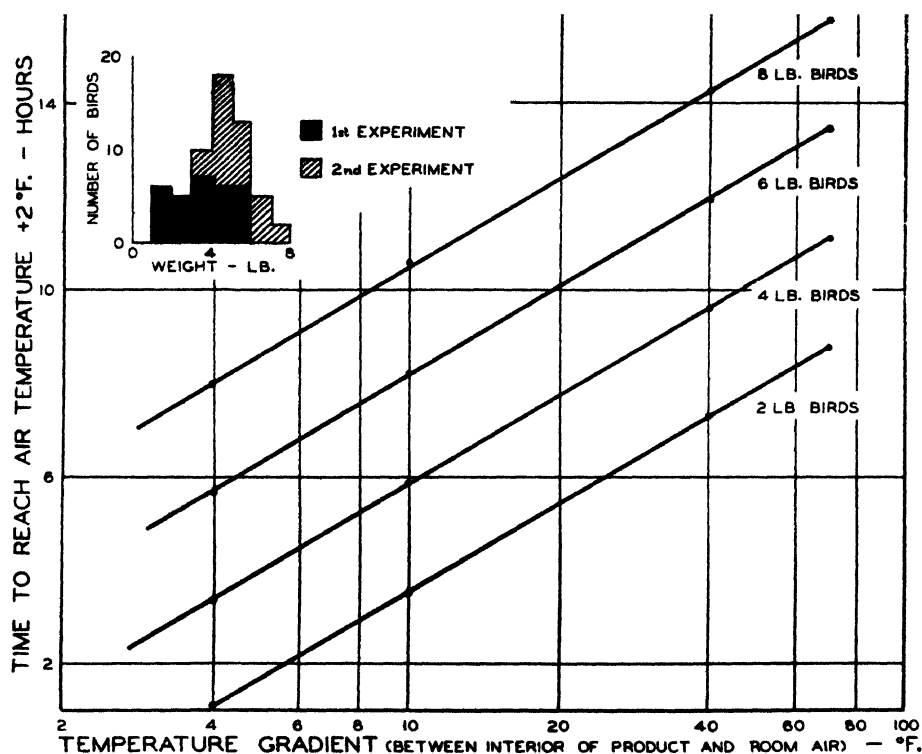
Fig. 2. Time required to cool birds of various temperatures and weights in air. (Insert shows weight distribution of birds used in establishing equations.)

temperature between the interior of the product and the air in the cooling room and has been plotted on a logarithmic scale to make the relation linear. The relatively large standard deviations, even after the effect of variable initial temperature and weight have been accounted for (Table 3), show the importance of other factors, such as shape of the bird, fat content, and deposition, in determining the time for cooling individual birds.

The mean weight of the birds used, the time required for cooling from their initial temperature to 1.1°C. (34°F.), and the standard

deviation of an individual bird from this mean time are reported (Table 3). Birds of 3.5 pounds in weight required a mean time of about five hours for cooling in air. A preliminary immersion in water at 7.2°C.(45°F.) before hanging in air at 0°C.(32°F.) had

TABLE 2

Analysis of Variance of Cooling Time in Air

Variance accounted for by	First experiment only		First and second experiments	
	Degrees freedom	Mean square	Degrees freedom	Mean square
Variable initial-product temperature..	1	36.758 [1]	1	37.328 [1]
Added effect of variable weight.........	1	8.998 [1]	1	157.348 [1]
Added effect of variable Wt.[2]......	1	4.715 [2]	1	0.000
Residual..	26	0.821	55	3.233

[1] Significant 1% point   [2] Significant 5% point

little effect on the time required to reach 34°F., although it did hasten cooling in the initial stages. Immersion in water at 32°F. for two hours before hanging reduces the time required to about 60 per cent of that required in air.

TABLE 3

Time Required for Precooling Poultry

Statistics	Birds precooled in air at 0°C (32°F.)		Birds cooled in water at 32°F for 2 hr then in air at 32°F	Birds cooled in water at 45°F for 2 hr. then in air at 32°F.
	1st exp only	1st and 2nd exp		
	lb.	lb.	lb.	lb.
1. Mean weight of birds...............	3.5	4.4	3.6	3.6
	hr.	hr.	hr.	hr.
2. Mean time.......................	5.0	6.9	2.86	4.9
3. Standard deviation (individual bird)...................	1.6	2.5	0.74	.....
4. Standard deviation (individual bird) after allowing for effect of variable initial temperature.......	1.1	2.4	0.69	.....
5. Standard deviation (individual bird) after allowing for effect of variable initial temperature and weight by linear equation........	1.0	1.8	0.57	.....

SHRINKAGE

Shrinkage was measured by weighing each bird separately on receipt at the cooling room and again 24 hours later at the time of packing. This made possible estimates of variability in shrinkage

between individual birds treated in the same way. Since the amount of shrinkage is determined by the factors that determine the rate of cooling, it was thought that the observed shrinkage might be related to the initial temperature and weight of the birds. When the results were analyzed, however, it was found that the variability in the shrinkage between individual birds was so great that it was impossible to establish these relations precisely.

The observed mean change in weight and the standard deviation for an individual bird are reported (Table 4). Two lots of birds were precooled in air under slightly different conditions and the

TABLE 4

Shrinkage of Poultry Precooled in Different Ways

Precooling conditions	Number of birds	Weight of birds	Mean change in weight	Standard deviation (individual bird)
		lb.	*pct.*	*pct.*
Convection cooled room, 32°F. Rel. hum. 70% at first, rising to over 80% for a few hours.	50	2-4	—0.24	0.12
	51	4-6	—0.22	0.09
Air stirred intermittently, 32°F. Rel. hum. initially 70%, rising to 75% for a few hours.	6	< 2	—1.10 [1]	0.40
	11	2-4	—0.78 [2]	0.15
	12	4-6	—0.41 [1]	0.15
Immersed in water at 32°F. or 45°F. for 2 hr. then hung in air as in experiment immediately above.	26	1.5-6	0.22	3.10

[1] Means significantly different.

results from each lot have been reported separately. Within each of the lots the birds have been classified according to their weight. The shrinkage observed in birds cooled by immersion in water at 32 and 45°F. for two hours before hanging in air is also reported for comparison. In this connection, it should be pointed out that the final weight was taken 24 hours after receipt at the room, when the birds were completely dried off and ready for packing.

The first experiment (Table 4) was conducted in a room at 32°F. without forced air circulation. The initial relative humidity was about 70 per cent and increased to over 80 per cent when the warm birds were placed in the room. At the time the final weights were taken the humidity was again about 70 per cent. No temperature observations were made on this group of birds. It is evident that the mean loss in weight was about one-fourth per cent and the loss in the two- to four-pound weight range was not significantly differ-

ent from that in the four- to six-pound weight range. Relative to this small loss the observed variations were quite large as shown by the standard deviations reported for individual birds. Some birds showed negligible weight losses while a few others showed losses in excess of one-half per cent.

The second experiment on cooling in air was made under the conditions described in discussing the precooling time. The slightly lower relative humidity with intermittent air circulation and the lower mean weight of the birds probably all contributed to a greater loss in weight, the mean value being about three-fourths per cent Under these conditions it was possible to demonstrate that the percentage of shrinkage decreased as the weight of the birds increased, the differences between the shrinkage of the different weight classes being statistically significant (Table 4).

Since the evaporation of moisture from the product also aids in cooling, it is of interest to determine what proportion of the heat was removed in this way under the two sets of conditions used. At one-fourth per cent shrinkage 100 pounds of product would lose one-fourth pound of water, equivalent to the removal of about 265 B.T.U. Since the specific heat of the product is about .8, the removal of this amount of heat would cool this mass of product somewhat over three degrees Fahrenheit. Product suffering one-half per cent shrinkage would be cooled about seven degrees F. by evaporation. Since the majority of the birds were cooled over 50 degrees F., it is obvious that evaporation does not contribute over about 15 per cent of the cooling at the most.

In the experiments involving immersion in water for two hours prior to hanging in air, the fowls showed a mean gain in weight of 2.2 per cent after hanging 22 hours in air and the surfaces were completely dry. This value was obtained after eliminating all the birds in which the leakage of water into the body cavity around the thermocouple leads was suspected. Nevertheless, the relatively large gain in weight, combined with the enormous variability between different birds, suggests that water may have entered the body cavity through other channels.

In conclusion it appears that a 24-hour precooling period in air, under conditions approximating those found in commerce, will cause an average shrinkage of about one-fourth per cent. Lower relative humidities than those used in these tests or forced air circulation will increase the shrinkage. As might be expected, shrinkage depends on the size of the product. Light birds suffer a greater shrinkage than heavy birds; those below two pounds may lose one per cent during cooling. Immersion in water for two hours prior to hanging

in air, results in a gain in weight of about two per cent, probably owing to the retention of water in the body cavity. The loss or gain of an individual bird deviates greatly from the mean values reported; some show negligible change while others may gain or lose over twice the average amount.

BACTERIAL CONTENT OF WATER USED FOR PRECOOLING

Where temporary immersion in water is used to hasten the cooling rate, it is possible that the water may also be a source of contamination and, by increasing the bacterial load on the surface of the product, may reduce its safe storage life in the chilled state, according to Lochhead and Landerkin (1935). Storage experiments at temperatures above the freezing point were not conducted nor was the bacterial content of product investigated, but some preliminary

TABLE 5

Bacterial Content of Water Used for Precooling

Date of sampling	Lot No.	Water at 0°C.(32°F.)		Water at 7.2°C (45°F)	
		Number of bacteria per ml of water		Number of bacteria per ml of water	
		Before immersion Log 10	After immersion Log 10	Before immersion Log 10	After immersion Log 10
1/10/36	1	2.2	2.3	3.6	3.4
2/10/36	2	2.1	2.4	4.1	3.8
5/10/36	3	2.4	2.6	4.4	4.5
6/10/36	4	2.4	2.7	7.2	7.3
9/10/36	5	2.6	3.0	5.6	6.0

measurements were made on the bacterial content of the water in the tanks used for precooling. Samples were taken immediately before and after immersing each lot of birds, and as the same water was used for all lots, the results also give some indication of the changes likely to occur on standing. These changes must be interpreted with caution, however, since the time interval between successive immersions and counts varied somewhat, and the temperature was not closely controlled during these intervals. The bacterial numbers were estimated by plating appropriate dilutions on Bacto-nutrient agar and incubating them for 48 hours at 20°C.(68°F.) before counting.

The logarithms of the bacterial numbers observed in the tanks at 0 and 7.2°C.(32 and 45°F.) are given (Table 5). The same water was used in both tanks and the higher initial count in the tank at 45°F. must be attributed to the growth which occurred during the period of temperature adjustment prior to making the first count.

It can be seen that the immersion of each lot of birds generally tended to increase the bacterial count slightly at both temperatures, but this increase cannot be regarded as serious in relation to the number originally present. It is also evident that the bacterial content of the water in the tank at 32°F. increased somewhat over the entire experiment, while the water at 45°F. increased markedly. In commercial practice the water would have to be changed frequently to maintain the required temperature, and this gradual increase would be unlikely to occur. At the same time these results indicate that a water temperature near the freezing point is desirable in order to keep the bacterial count as low as possible.

EFFECT OF PRECOOLING ON BLOOM AND APPEARANCE

Some operators feel that the temporary immersion of poultry in water may be detrimental to the bloom or appearance. This point was investigated by packing five boxes, each of which contained six birds precooled by immersion in water for two hours followed by hanging in air, and six birds precooled in air only. Since bacterial activity limits the storage life to a few weeks at temperatures above the freezing point, they were stored in the frozen state at —13.6°C. (7.5°F.). The birds were examined at the time of packing and periodically thereafter for 139 days, when the surfaces were so badly desiccated (freezer-burned), that further observations were useless. The usual commercial boxes and wax-paper liners were used throughout.

Two inspectors examined and scored the individual birds for bloom at each examination. Five grades of bloom were defined in advance and each allotted a whole number which increased with the quality. As the breasts and legs were scored separately, a score of ten was possible for each bird. These individual scores were later summed for the six birds precooled by each method in each box. These summed scores were used in making all subsequent calculations and comparisons.

Before presenting the results it is necessary to discuss the arbitrary nature of such subjective observations. It was impossible to compare individually each of the 60 birds used in the experiment. In the first place the birds were frozen and could not be moved and classified separately. Secondly, the five boxes of birds varied in net weight from about 20 to 63 pounds and, as the quality and finish is somewhat dependent on the weight, it was difficult to assess the differences in bloom among birds that varied so greatly in over-all quality. In consequence, each box was scored as an individual lot, and the five boxes may be regarded as replications. In scoring, the

practice was to select at each examination one or two birds in each box that seemed to be representative of one of the defined scores. This bird or birds, was then taken as a standard in scoring the remaining birds. By this method it was possible to place certain birds in an intermediate position between two defined grades, and these birds were given the score for the lower grade plus a half.

It is not necessary to consider the individual scores by birds or boxes in detail. As the birds were chosen at random for this investigation, the over-all quality of the product varied considerably between birds, between lots precooled in water and air and packed in the same box, and between boxes. This was reflected in the scores at all examinations. The general trend was for bloom to decrease

TABLE 6

Comparison of Precooling in Air and Water on Subsequent Bloom of Birds in Frozen Storage at —13.6°C.(7.5°F.)

Box No. and weight (net)			Ratio bloom scores of birds precooled in water and in air				
			Initial after freezing	After 31 days' storage	After 65 days' storage	After 92 days' storage	After 138 days' storage
	lb.	*oz.*					
1	19	13	110	115	116	104	129
2	35	8	106	103	102	101	112
3	44	6	113	118	91	100	105
4	51	10	91	96	91	92	72
5	62	14	100	92	99	100	109

Differences	Degrees freedom	Mean square
Within examinations	20	150.5
Between examinations	4	53.8

with increasing time in storage. There was also a tendency for the boxes having the poorest bloom initially to decrease most. The scores allotted at one examination were consistently higher than those allotted on the previous and subsequent examinations, and the differences between the several boxes were also less marked on that occasion. This result can be attributed to the subjective nature of the scoring, and casts doubt on the significance of the quantitative estimates of the decrease of bloom with time.

Since birds precooled by the two methods under test were packed in the same box, and all compared with a bird in that box as a standard, the effect of the two precooling treatments can be considered independent of variations in the inspectors' judgment affecting comparisons between boxes or between inspections. The scores of the birds precooled in water were expressed as a percentage of those

precooled in air for all boxes and observations (Table 6). The departure of this ratio from 100 per cent in the initial examination merely indicates the variable quality of the birds precooled by the two methods.

These results were treated statistically by computing the variance within and between examinations. The variance within examinations may be taken as an estimate of the "experimental" error, while that between examinations, i.e., with time, would show any differential effect of storage on the birds precooled by the two methods. From the results it is obvious that the variance within examinations, which results from the variable initial quality of the product and the inspectors' judgment, far exceeds the differences arising during storage (Table 6, lower part). It is therefore concluded that if any differential effect does occur, it is negligible compared with the variations ordinarily observed between birds similarly treated. This conclusion is in agreement with the opinion of practical inspectors who examined the product at the final inspection and stated that the evident differences were due to the quality of the birds themselves rather than to the different treatments.

SUMMARY

Equations are given relating the initial-product temperature, air temperature, and weight of the bird to the average time required for cooling to any required temperature above the freezing point. The importance of certain unknown properties of the product in determining the time required for precooling is demonstrated by the relatively large residual variance, after accounting for the effect of temperature gradient and weight by the above equations.

Immersing the birds in water at 7.2°C.(45°F.) for two hours prior to hanging in air at 0°C.(32°F.) has little effect on the over-all time required for cooling. A similar treatment using water at 32°F. reduces the time to about 60 per cent of that required in air alone. The loss in weight during cooling in air varies, on the average, from one-fourth to one-half per cent, depending on the atmospheric conditions. Individual birds deviate considerably from these mean values. Immersion in water for two hours results in a mean over-all gain in weight of extremely variable magnitude. The bacterial content of the water used for immersing the birds was much higher in the tank at 45°F. than in the tank at 32°F. No evidence was obtained to indicate that temporary immersion in water during precooling had either a detrimental or beneficial effect on the retention of bloom during subsequent storage in the frozen state.

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THE QUALITY OF FRESH, FROZEN AND STORED HALIBUT AS DETERMINED BY A TASTING PANEL

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As reported in a News Item in Progress Report No. 32, a Fish "Tasting Panel" consisting of six individuals meets every two weeks at this Station to estimate the quality of cooked halibut samples given various treatments.

The individuals for the panel were selected from a dozen or more volunteers who submitted to a preliminary test to determine their sensitivity of taste and smell. From this preliminary test, although it was found that all the women secured higher marks than the men, for the regular panel the three men and three women securing the highest marks were selected, the remainder being reserved for substitues.

The object of the panel was primarily to correlate any chemical, physical and electrical changes in halibut muscle as found by the various workers investigating the quality of our Pacific coast halibut, with changes in characteristics of the cooked muscle, in the hopes that some quick means could be discovered to determine the staleness or freshness of halibut. The studies have been extended to frozen and cold stored halibut to determine the effect of different rates of freezing and different storage temperatures on the ultimate or cooked product.

The experiments were planned to eliminate as many variables as possible other than those to be studied. For example, in the frozen fish experiments, fish from the same grounds and caught at the same time were used for the samples and care was taken to have the samples for each fortnightly test prepared from the same fish. Therefore, except for the slight differences due to variations in different parts of the same fish, any other differences would necessarily be attributed to treatment only. Obviously absolute controls could not be kept for the cold storage experiments. The best that could be done under the circumstances was to use as control samples parts of fresh fish obtained from the market at the time of the particular tests.

In the freezing and storage experiments, samples were rapidly and slowly frozen and stored at 14° F. and at -13° F. The samples were put up in 1-lb flat cans and sealed, after which for rapid freezing some were immersed directly into -13° F. calcium chloride brine which caused the temperature at the center of the cans to pass through the critical zone (32° to 23° F.) in approximately 22 minutes. For slow freezing the cans were merely placed in a storage room held at -13° F. The temperature at the center of the cans in this case was found to pass through the critical zone in 6½ hours.

The samples for the fresh (unfrozen) fish experiments were obtained through the cooperation of fishermen. In order to obtain fish of known post mortem history a few fish from each day's catch were marked and set apart but were otherwise treated in the same manner as the bulk fish. In this way fish stored in ice for different lengths of time were compared.

The procedure in the cooking tests has been to cut the samples into about 1¼-inch cubes, immerse these in salted milk, roll them in dried bread crumbs, place them on an oiled pyrex platter and cook them 14 minutes in an electrically heated oven in which the temperature was brought up to 570° F. prior to the insertion of the samples. After cooking, the numbered samples are placed on pre-heated petri dishes which are kept warm during the tests by a special hot plate. The samples are

graded for texture, cook, appearance, flavour and odour by arbitrary qualifying terms suggested by the members of the panel themselves.

Experiments of the above nature are not productive of definite or clear-cut results in the first place because of the human factors involved in judging the samples; secondly because in most cases the differences to be determined are extremely slight and may therefore be easily misjudged. The attitude adopted in appraising the results has been to attribute inconsistencies to differences that are too slight to be worthy of recognition, or in other words to over-zealousness on the part of the testers. This over-zealousness has been brought to the attention of the testers from time to time by control tests where more than one control has been used in the same test. Infallible testers would invariably give all the controls equal grading; consequently these control tests not only give an indication of the reliability of the grading, but they permit the testers to estimate to what extent their gradings are to be modified for consistency.

The results obtained with the regular tasting panel have been somewhat difficult to interpret as wherever possible they have been supplemented with results from similar tests performed with some thirty families representing a cross-section of the city. By increasing the number of samples in this way the results can be finally treated statistically.

The experiments are not completed yet and the computations cannot therefore be made, but a few results are given below for illustrative purposes.

Expt. 1—Unfrozen fish stored in ice 2 days as compared with 9 days.
10 families preferred the 2 day old fish,
4 families preferred the 9 day old fish,
1 family declared them the same.

Expt. 2—Unfrozen fish stored in ice 2 days as compared with 7 days.
The results were equally divided.

Expt. 10—Frozen fish stored in ice 3 days prior to freezing, one half of each fish kept unfrozen as absolute control.
10 families preferred the unfrozen samples,
6 families preferred the frozen samples,
5 families discerned no difference.

Expt. 12—Same as experiment 10 except the fish were stored in ice 8 days prior to freezing.
3 families preferred the unfrozen samples,
2 families preferred the frozen samples,
14 families declared them the same.

Expt. 15—Control test where samples from the same fish were sent out untreated and should therefore be the same.
14 families reported differences,
4 families reported no difference.

Results of numerous tests similar to those outlined above, together with the remarks turned in by the various testers, lead to the following observations: (1) There are considerable differences in cooked halibut of identical post mortem history even though of the same weight and caught on the same banks at the same time. (2) Judging from our tests, the majority of people prefer fresh halibut. That is, halibut that is still in rigor or in which rigor has but shortly passed off. After 7 days' storage in ice, halibut begins to lose its appeal and by 11 days is usually described as slightly "off" in flavour. (3) Halibut "ages" just as does beef and game, the prime age being between 5-7 days' storage in ice. Although some testers prefer fish in which the enzymes have produced the stronger or "off" flavours, most prefer fish under 7 days' storage in ice. (4) After cooking, halibut that was properly frozen is very difficult to distinguish from unfrozen samples. The appearance, odour and flavour are similar but careful comparisons usually show that the frozen fish is slightly firmer and sometimes drier than the unfrozen samples. The differences are often less than those that may be found in different unfrozen fish. (5) Many people prefer firm dry fish and to them frozen halibut has
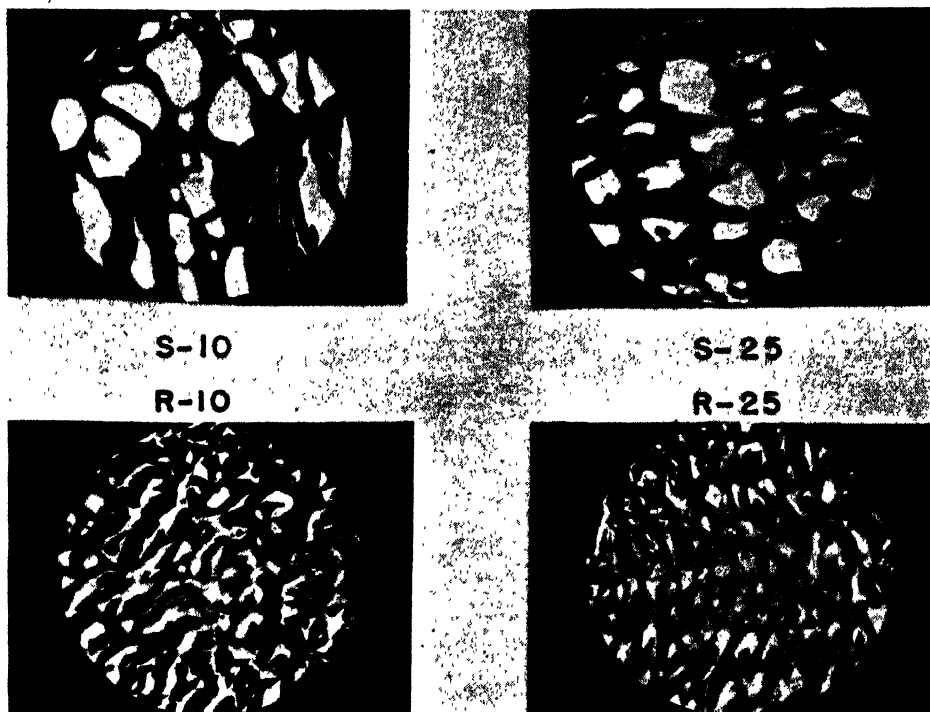
Figure 1

a greater appeal than unfrozen halibut. (6) Properly frozen fresh halibut has a greater appeal than the slightly "off" flavoured unfrozen halibut, which implies that it would be better to supply our remote markets with all frozen halibut than to ship unfrozen fish that obviously cannot reach the consumer in an appealing condition.

The results of the investigation into the effects of freezing rate and storage temperature on the keeping qualities of frozen halibut are somewhat difficult to interpret but a great deal of interesting information has been obtained from them, and every effort is being made to assist the tasters in their grading, in order to permit definite conclusions being drawn.

All frozen and stored samples are photomicrographed prior to the tests to discover changes that may occur in the size of ice crystals at the different storage temperatures. The samples are also carefully examined in the frozen and thawed states. Up to the cooked stage there are marked differences between rapidly and slowly frozen halibut and some minor differences in the samples stored at different temperatures, but after the cooking the differences are greatly reduced, making the grading very difficult.

The photomicrographs shown in figure 1 are those taken of a series of samples tested after 3 months in storage. All the samples are from the same fish. Sample S-10 was frozen slowly (taking 6½ hours for the temperature to pass through the critical zone) and stored at 14° F. Sample S-25 was frozen at the same rate but stored at -13° F. Sample R-10 was rapidly frozen (taking but 22 minutes for the temperature to pass through the critical zone) and stored at 14° F.; while sample R-25 also rapidly frozen was stored at -13° F. The difference in size of crystals is very marked between the slowly and rapidly frozen samples, but is slight between samples frozen at the same rate but stored at different temperatures. It appears however that even at three months the crystals are somewhat larger in the samples stored at the higher temperature.

Examination of the samples in the frozen state reveal differences as marked as those shown by the accompanying photomicrographs. In the slowly frozen samples the ice crystals are apparent to the unassisted eye and after thawing, the muscle is spongy. Liquid may be squeezed from the samples quite easily and most of this liquid may be re-absorbed when the pressure is released. In both the frozen and the thawed state the slowly frozen samples have a dull watery appearance quite different from the rapidly frozen samples. These latter appear whiter or more opaque and the muscle is not spongy in appearance though liquid may be squeezed from it much as with the slowly frozen samples. But surfaces of the rapidly frozen samples appear more glossy than do those of the slowly frozen samples, though they are not as glossy as those in unfrozen fish.

Judging by the appearance of the sample in the frozen and in the thawed states, rate of freezing appears to be the most important factor in preserving the original characteristics of halibut because samples examined after 14 months in storage show the rapidly frozen specimens stored at the higher temperature to be much superior in appearance to those frozen slowly but stored at the lower temperature. The findings of the tasters, however, do not bear this out, as is shown by the results of one test given in the accompanying table.

Sample	Treatment	No. of 1st grades received	No. of 2nd grades received	No. of 3rd grades received	No. of 4th grades received
R-25	Rapidly frozen stored at -13° F.	31	14	4	4
S-25	Slowly frozen stored at -13° F.	13	17	18	5
R-10	Rapidly frozen stored at +14° F.	7	14	14	18
S-10	Slowly frozen stored at +14° F.	2	10	18	23

The above table gives the results of a 5-month test carried out in the winter when no controls could be obtained. During this time sample S-25 stood up much better than did sample R-10, showing that temperature of storage is more important in maintaining quality than is rate of freezing. Rate of freezing is also very important, however, because at both storage temperatures the rapidly frozen samples graded higher than did the slowly frozen ones.

The test tabulated above was simplified through the lack of a control, therefore the results are much more definite than those obtained with controls. In none of the experiments, however, has a definite break occurred in the gradings to show when the effect of the higher storage temperature is first noticeable.

Slowly frozen fish even if stored at 14° F. for 14 months can be made into very tasty dishes but it requires very careful handling. In the hands of careless or inexperienced cooks its appeal may be entirely lost. Our experiments show that the lower temperature of storage is much superior but they do not show yet how long, if at all, the higher temperature may be safe. The obvious safe method for best results even for very short storage periods is to employ not only the quickest economical method of freezing but also storage temperatures in the neighbourhood of -13° F. or lower.

Acknowledgment

SOME OBSERVATIONS ON THE FUMIGATION OF APPLES WITH METHYL BROMIDE[1]

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Methyl bromide was first reported as an insecticide by Le Goupils (9) in 1932. More active investigations have been undertaken by entomologists during the last two years with this fumigant because of its toxicity to insects, non-explosive properties and facility of volatilization. Mackie and Carter (11 and 12) and Fisk and Shepard (4) have indicated in their publications the various products on which methyl bromide can be used.

Because of the low solubility in water of this fumigant there is some reason to believe that it may be used to destroy insects feeding internally in plant tissues, without damaging these tissues. Hence investigations were carried out to study the toxicity on apple feeding insects as well as the possible injury which might result to the apple.

EXPERIMENTAL PROCEDURE

Material Used and Method of Sampling

Previous to 1937 the main considerations were given to effect on the insect pests. The findings that the fumigation treatment necessary to kill the insects was harmful to the fruit under certain conditions necessitated a thorough investigation into fruit tolerance to the fumigation methods involved.

For this purpose McIntosh apples were used. They were grown on S. R. Jack's orchard at Châteauguay, P.Q. Twelve trees free from injury and disease and bearing a moderate crop were selected. All samples of fruit used in the experiment contained an equal number of apples from each tree.

The crop off these trees was picked at three different dates giving three maturities of fruit, one earlier, another at the same time, and another later than the recommended commercial picking maturity. One-half of each of these picks was stored at 39° F. and the other at 32° F., representing the extremes of ordinary commercial storage temperatures. Both of these groups were divided into four equal parts to be treated at four different times after picking, *i.e.*, T_0—treated immediately, T_2—two weeks, T_4—four weeks and T_6—six weeks after picking. Each of these last groups was made up of ten ½-bushel hampers of approximately 60 apples in each. Each individual sample was exposed to 1 of 10 different fumigation treatments.

Method of Fumigation and Handling

All samples of fruit, except when being fumigated, were stored at the Horticultural Division, Central Experimental Farm, Ottawa. Each group of apples fumigated at the same time was sent to the Dominion Fumigation Station, Montreal, where the necessary fumigation treatments were made as shown in Table 1.

Previous to the actual fumigation treatment, care was taken to see that the internal temperature of the apples was up to 60° F. so that the insects would be in a state of susceptibility to the fumigant. The fruit was then placed in a vacuum fumigation chamber of 30 cu. ft. capacity. This chamber was held at 80° F. by means of steam heating.

When the apples were placed in the vacuum chamber the required vacuum was created. The desired amount of fumigant was then allowed to flow into the evacuated chamber. The amount of gas was measured by increase in pressure by the usual method based on the simple gas law formula $PV = RnT$.

The fruit was exposed to the fumigant for 2 hours in the standard treatment, which period is 30 minutes in excess of the usual standard exposure time. At the end of this time a routine "air washing" was made with 2 successive vacua of 3″ absolute pressure following the restoration of the pressure in the chamber to 27″ absolute pressure. An electric fan in the chamber running 15 minutes on and 15 minutes off was used for gas circulation.

In previous experiments in 1936 the standard treatment with a proprietary fumigant containing approximately 7% methyl bromide and 93% carbon dioxide resulted in injury to the apples. In order to determine which phase of the technique was causing the damage the variations and components of the standard treatment were used as shown in Table 1.

The apples used for analytical work were those in the series receiving treatment No. 7 and kept at a storage temperature of 32° F.

Determination of Methyl Bromide Traces

The technique as finally adopted was based on a method designed for the determination of ethyl bromide in physiological material by F. L. Hahn (6). The sample under analysis was heated with aqueous vapor and air, the mixture being drawn by aspiration through a hot quartz tube. The organic bromide was decomposed and changed to hydrobromic acid. The hydrobromic acid was trapped in 2 gas washing bottles fitted with fritted glass bubblers and containing $N/2$ KOH.

Preparation of Sample

Five apples were chosen at random from the cold storage room, each apple being cut into three sections. The core sections were combined and analysed as a composite. The inner and outer cortex sections were similarly treated using suitable aliquots. To avoid loss of methyl bromide the apples were cut into moderately large pieces and quickly placed in tared flasks closed by rubber stoppers wrapped in tin foil and kept on ice. The aspiration was continued for one hour, when the gas washing bottles were washed into 200 m. volumetric flasks. The quantitative determination was carried out as described under "Total Bromine".

Table 1.—Synopsis of Fumigation Treatments of McIntosh Apples*

Treatment No.	Reference name	Gas used	Dose in lbs. per 1000 cubic feet	Initial vacuum (in inches absolute pressure)	Absolute pressure after introduction of dose	Post-dosage treatment	Absolute pressure during fumigation
1	Control	No treatment					
2	"Vacuum control"			3"		Maintained	3"
3	"CO₂ alone"	Carbon dioxide	107	0 7"	29.5"	Maintained	29.5"
4	"CO₂ and vacuum"	Carbon dioxide	32.5	3"	11.7"	Maintained	11.7"
5	Methyl bromide at atmosphere.	Methyl bromide	2 5	25"	25 3"	Dissipated with air	29.5"
6	Gas mixture at atmosphere.	Methyl bromide and carbon dioxide.	CH₃Br 2.45 CO₂ 32.55	18"	27"	Dissipated with air	29 5"
7	Methyl bromide standard	Methyl bromide alone	2.5	3"	3.3"	Dissipated with air	27"
8	Gas mixture standard	Methyl bromide and carbon dioxide	CH₃Br 2 45 CO₂ 32.55	3"	12"	Dissipated with air	27"
9	Methyl bromide and vacuum.	Methyl bromide	2.5	3"	3.3"	Maintained	3.3"
10	Gas mixture and vacuum	Methyl bromide and carbon dioxide	CH₃Br 2.45 CO₂ 32.55	3"	12"	Maintained	12"

* Exposure period two hours.   Vault temperature maintained at 80° F.   Dosages and pressures originally applied in metric units.

Recovery of Methyl Bromide

From a small cylinder the methyl bromide was cautiously released through towers of N. KOH and conc. H_2SO_4 and allowed to condense in clean dry test tube buried in an ice-salt mixture. Using a chilled Mohr pipette a small amount of the condensate, which was clear and colorless, was measured into a chilled volumetric flask containing cold alcohol, 95% by volume. Suitable aliquots of this solution were used as in a determination and recoveries obtained as shown.

Added mgms.	Recovered mgms.	Percentage
1.386	1.272	91.7
1.386	1.314	94.7

Determination of Total Bromine

The technique for ashing the samples was essentially that used by Francis and Harvey (5), with modifications by Neufeld (14). The oxidation of bromides to bromate and subsequent iodometric titration was based on the method of Van der Meulen (16) and modified by Kolthoff and Yutzy (8).

According to Van der Meulen, bromides are quantitatively oxidised to bromates by $2\frac{1}{2}$ times the theoretical amount of hypochlorite in the presence of borate buffer and half saturation with NaCl.

$$3KClO + KBr \rightleftharpoons KBrO_3 + 3KCl$$
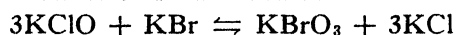
The reaction goes completely to the right if the oxidation is carried out at 85° C. and the excess hypochlorite is removed by hydrogen peroxide, which is in turn removed by boiling. Adding bromates to iodides in acid solution releases iodine, equivalent to 6 times the original amount of bromine.

$$HBrO_3 + 6HI \rightleftharpoons HBr + 3H_2O + 3I_2$$

D'Ans and Höfer (3) modified the technique using sodium dihydrogen phosphate in place of borate buffer, and sodium formate to remove excess hypochlorite.

Kolthoff and Yutzy found that for the determination of 25 ml. of 0.001 M bromide or less, the addition of sodium chloride was unnecessary.

TABLE 2.—RECOVERY OF ADDED POTASSIUM BROMIDE*

Added bromine, mgms.	Normality of thiosulphate	Thiosulphate used ML	"Blank" ML	Recovery MGM	Bromine percentage
0.2838	0.00978	2.42	0.26	0.2814	99.1
0.2838	0.00975	2.58 ⎫	0.32	0.2883	101.6
		2.50 ⎬			
0.2838	0.00975	2.40 ⎰	0.27	0.2792	98.3
		2 44 ⎭			
0.2270	0.00978	1.97	0.25	0.2241	98.7
0.2270	0.00978	1.99	0.25	0.2267	99.8
0.1700	0.00978	1.55	0.25	0.1693	99.6
		14.50 ⎫			
0.9460	0.00510	14.50 ⎬	0.72	0.9353	98.8
		14.44 ⎭			
		4.19 ⎫			
0.1810	0.004972	4 24 ⎬	1.51	0.1782	98.4
		4.16 ⎭			

* These values were determined on potassium bromide solutions.

Preparation of Sample

Five apples, chosen at random from the 32° F. storage room, were put through a meat grinder and thoroughly mixed. Portions were weighed out for moisture determinations.

Forty grams, weighed to the nearest decigram were ashed in nickel crucibles according to the reference above. The final ash extract was made to volume in 50 ml. flasks. Suitable aliquots were oxidised and the iodometric titrations completed. Blanks on the reagents were run daily. The thiosulphate solution was standardised daily also.

The recovery of bromine on adding potassium bromide to fumigated and control apples gave values of 98.7, 98.4% and 99.8 and 99.5%.

EXPERIMENTAL RESULTS

Toxicity to Insects Infesting Apples

Among insects liable to infest apples, Mackie and Carter (11) found, in 1937, that methyl bromide applied alone at the rate of 2.5 pounds per thousand cubic feet in dissipated vacuum treatment was completely toxic to larvae and pupae of the codling moth *Carpocapsa pomonella* Linn., in pears. Lindgren (10) also found, in 1936, that a proprietary fumigant containing methyl bromide and carbon dioxide in mixture was toxic to the larvae of the same insect buried in burlap sacks. These results were confirmed under Canadian conditions by McLaine and Monro (13). Mackie and Carter (11) also found the pure methyl bromide effective against the San José scale *Aspidiotus perniciosus* Comstock.

Fumigation of the Apple Maggot

Experiments conducted in 1936 against the apple maggot *Rhagoletis pomonella* Walsh infesting apples showed promise, and a more careful investigation was undertaken in 1937. An attempt was made to fumigate the apples in the stages of maturity in which they were likely to be shipped. In this condition the larvae are usually small, as the state of maturity of the apples influences the development of the maggots within the fruits. The maggots do not usually attain maturity and prepare to emerge from the apples until the fruit is overripe. Apples from Simcoe, Ontario, at different stages of maturity were forwarded to Montreal under the direction of Mr. W. A. Ross of the Entomological Laboratory, Vineland Station, Ontario.

For the most part these apples contained maggots from eggs which had been laid during the latter half of July, 1937. From what is known about the period of egg laying in the Simcoe orchards, and of the stage of development of the insect in both immature fruit and mature fruit fit to ship, it may be assumed that at the time of fumigation the apples contained a preponderance of small maggots and probably a few unhatched eggs. The apples were fumigated in the experimental vault of 30 cubic feet capacity as soon as possible after their arrival in Montreal, being subjected to the standard dissipated vacuum treatment for different periods of time as indicated in Table 3. The apples were then placed in metal cans which were examined periodically for emerged mature larvae, as these usually drop from the apples and congregate on the bottom of the cans. After the maggots ceased emerging, all the apples were cut open to find any further

TABLE 3.—TOXICITY OF METHYL BROMIDE TO LARVAE OF THE APPLE MAGGOT *Rhagoletis pomonella* WALSH*

Host variety	Origin	Date fumigated	No. of apples per treatment	Larval survival in apples					Emergence period of larvae from apples
				Treatment exposure in hours				Control	
				2	1½	1	¾		
Immature Wealthy	Sent from Simcoe, Ont., Aug. 6, 1937.	Aug. 9	22	0	0	0	0	54	Aug. 31 – Sept. 4
Mature Wealthy	Sent from Simcoe, Ont., Aug. 20, 1937.	Aug. 25	20	0	0	0	0	7	Sept. 1 – Sept. 4
Fameuse	Sent from Simcoe, Ont., Aug. 23, 1937.	Aug. 25	20	0	0	0	2	51	Sept. 7 – Sept. 22
Fameuse	Sent from Simcoe, Ont., Aug. 30, 1937	Sept. 1	35	0	0	0	12	116	Sept. 9 – Sept. 29

* Methyl bromide applied at the rate of 2.5 pounds per 1000 cubic feet. Initial vacuum 3 inches of absolute mercurial pressure reduced by air after the introduction of the dose to 27 inches absolute pressure. Vault temperatures 80° F. to 82° F. Fruit temperatures 75° F. to 78° F.
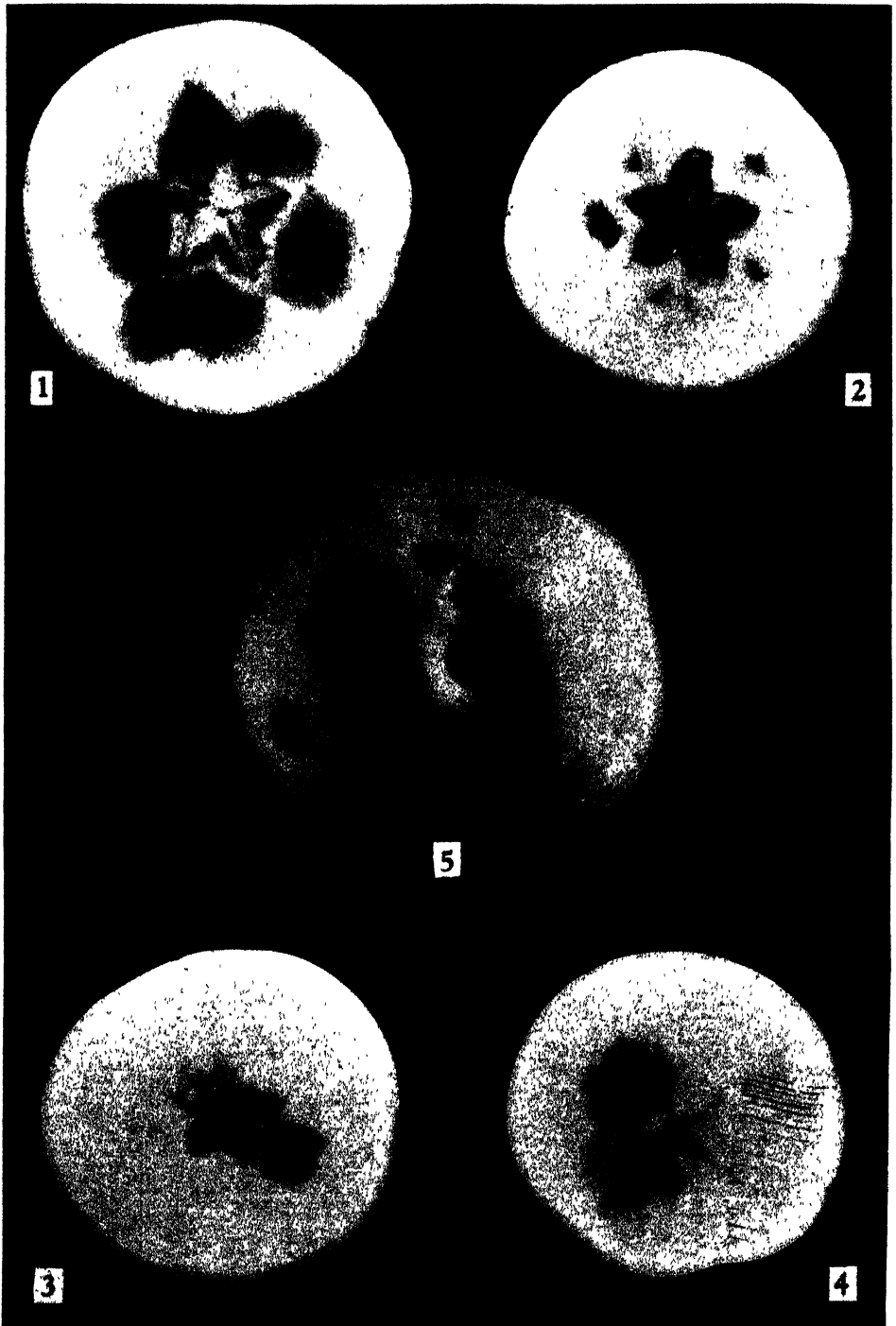
FIGURE 1.   Methyl bromide injury in McIntosh apples.   1, 2, 3 and 4 illustrate internal injury and 5 external.

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pupae which had formed inside.   From the results summarised in Table 3 it would appear that the standard exposure period of 90 minutes is well within the limits of complete toxicity to the maggot in the stage of development found in apples at the time of shipping.

In addition to the toxicity of this treatment to any eggs of the insect which may have been present, other preliminary experiments have also indicated that it is effective against the eggs.

Cause of Injury to Apples

It has been shown that a method of fumigating apples, which has proven successful in destroying the infesting insects, has in several instances damaged the apple tissues.   Figure 1 shows the external and internal injury found in apples after being exposed to methyl bromide fumigation treatment.

The data on which all fruit injury is based were obtained by examining the fruit between January 11th and 26th, 1938.   At this time other storage disorders were just beginning to develop.   Hence it was reasonable to assume that the point in storage had been reached at which fumigation injury had a maximum time to develop without being confused by other disorders.   Half of each sample was examined on removal from storage. The remaining half was examined after one week's exposure to ripening conditions of 60° F. temperature and 90 to 95% relative humidity.

All injury has been classified into three types according to intensity— slight, moderate and severe.   The percentage of apples affected with each type of injury was the basis for calculation.   An injury index figure was then calculated by multiplying the percentage of slight by 1, moderate by 2 and severe injury by 3.   Thus all calculations throughout this paper of injury index are based on a maximum of 300.

TABLE 4.—MEAN METHYL BROMIDE INJURY INDICES FOR THE VARIOUS
FUMIGATION TREATMENTS*

Fumigation treatment	Internal Injury	External Injury
1. Control	0	0
2. Vacuum alone	0	0
3. 100% CO_2	0	0
4. CO_2 + vacuum	0	0
5. CH_3Br no vacuum	29.9	1.0
6. CH_3Br gas mixture no vacuum	32.6	0
7. CH_3Br standard	27.7	1.3
8. CH_3Br gas mixture, standard	14.1	3.1
9. CH_3Br vacuum sustained	153.7	3.5
10. CH_3Br gas mixture vacuum sustained	15.8	2.7

* 1200 apples used for each calculation.

The mean injury index figures for all samples of fruit treated with each of the 10 treatments are shown in Table 4.   From this table it is obvious that methyl bromide must be present in the fumigation treatment to cause the injury.   It is interesting to note that the vacuum treatment and the CO_2 treatment in themselves result in no visible injury.   One unfortunate feature is that most of the injury by far is internal rather than

external. Thus it is only by cutting the fruit that a true estimate of the injury can be made.

By further consulting Table 4 it is seen that considerable variation exists according to the manner in which the methyl bromide is applied. A very important consideration is that there is no advantage in not using the vacuum for fumigant penetration. The extent of injury resulting at atmospheric pressure is just as great as when the vacuum treatment is employed (compare treatments 5 and 7).

In all cases where the vacuum is employed there appears to be an appreciable reduction in methyl bromide injury by the use of CO_2 as compared to the same treatment without CO_2. The fact that the presence of CO_2 reduces the injury in the presence of the vacuum rather than without eliminates the possibility that the injury reduction was caused by more efficient control of the methyl bromide concentration.

Development of Injury after Removal from Store

Some storage disorders in fruit are not apparent immediately after removal from the store, but develop after exposure to ripening temperatures. This was not true in the case of the methyl bromide injury except in the case of No. 9 treatment (CH_3Br and sustained vacuum). The extent of injury in fruit exposed to this treatment, which incidentally was far more severe than necessary, increased to a large extent after a week's exposure to ripening conditions.

TABLE 5. METHYL BROMIDE INJURY INDICES ON REMOVAL AND ONE WEEK LATER FOR FIRST PICK OF APPLES FUMIGATED WITH No. 9 TREATMENT

Temperature	Time elapse between picking and fumigation	On removal from store	One week of ripening
39° F.	0 weeks	148	300
	2 weeks	300	300
	4 weeks	40	136
	6 weeks	96	192
32° F.	0 weeks	178	264
	2 weeks	58	220
	4 weeks	0	18
	6 weeks	0	160

The fact that there should be more injury visible after one week's exposure to ripening conditions is not alarming. The fact, however, that in some cases no injury was apparent on removal and the injury index reached 160 in one week is hard to understand. It was only with the No. 9 treatment that this occurred.

The important feature is, however, that under any of the fumigation treatments which may be recommended, the amount of injury found on removal from store is a true criterion of the full extent of the damage.

The Effect of Storage Temperature

If the methyl bromide injury were a physiological one it was thought possible that the temperature of storage both previous to and after fumigation might have some influence on the extent of injury. It has been found

that McIntosh apples develop low temperature disorders at 32° F. which do not occur at 39° F. If temperature did influence the injury from a physiological standpoint it would be brought out by storing the apples at these two temperatures.

The results failed to reveal any significant difference in the extent of the injury. The mean methyl bromide injury index for treatment No. 7 at 39° F. was 83.0 and at 32° F., 87.0. For treatment No. 8 at 39° F. the mean was 40.5 and at 32° F., 44.0. Variables other than temperature showed such a great variation that these differences in temperature treatment were not significant. So it may be assumed that if temperature of storage does affect methyl bromide injury slightly, the cause is indirect rather than direct.

Effect of Maturity

It has been shown by Blackman (1) that apples pass through a definite metabolic drift both previous to and during ripening. Since this is true it is possible that apples may be more resistant to methyl bromide toxicity at one point in their ontogeny than at another.

If a variation in tolerance exists during the picking season it should be shown in a study of the injury found in the three maturities of apples represented in the experiment. The mean methyl bromide injury indices for all apples treated immediately after picking are as follows:

These figures clearly illustrate that apples picked as in maturity 2 are much more resistant to methyl bromide injury than are the earlier or later pickings. Fortunately the second maturity represents the stage at which the apples should be picked for best storage results.

Maturity	Mean of all apples treated	Mean of apples treated immediately
1	29.8	77.4
2	14 8	23.1
3	24.4	60.3

Effect of Delay between Picking and Fumigation

In the previous section it has been shown that the maturity of the fruit plays an important rôle in methyl bromide tolerance. However, it does not completely explain larger differences in the injury found in some samples.

If we assume that maturity 1 was at a stage of maturity designated by the letter A and maturity 2 and 3 by B and C, respectively, then maturity 1 would tend to proceed towards B, and maturity 2 toward C after picking. The injury then should decrease in maturity 1 after picking and increase in maturity 2 if maturity of the fruit were the sole consideration. Similarly maturity 3 might be expected to decrease in methyl bromide tolerance. This was not the case.

It was found that the third picking of fruit suffered much less if they were fumigated after a certain length of time in storage. If maturity were the only factor involved it is unreasonable that these fruits would behave in this way since the apples would tend to approach a more mature stage. It was found that all maturities behaved in this way, *viz.*, an increased tolerance to methyl bromide after picking.

The mean methyl bromide injury indices for all fruits treated with methyl bromide (3,600 fruit for each mean) at different time intervals was as follows:

Treated immediately	53.6
2 weeks after picking	20.3
4 weeks after picking	11.9
6 weeks after picking ∙	5.3

It is obvious from these figures that the time elapse between picking and fumigation is a very important factor in avoiding methyl bromide injury.

Since these figures include treatments which were extremely severe it would be wise to consider the amount of injury involved with treatments of a standard nature. For this purpose the mean injury indices for treatments 7 and 8 fumigated at various intervals after picking are shown:

Treated immediately	23.3
2 weeks after picking	4.3
4 weeks after picking	3.5
6 weeks after picking	0.0

From these figures it can readily be seen that if the apples were allowed to remain in storage for two weeks, the amount of injury was reduced to a very slight amount. When a six-week period elapsed between picking and fumigation, the injury was completely avoided.

From the preceding data it has been shown that both the maturity and the time-elapse between picking and fumigation are important in decreasing the amount of fumigation injury. The next point to consider is the correlation between these two important factors. In Table 6 are shown the trends of injury both by maturity- and time-elapse between picking and fumigation.

TABLE 6.—EXTENT OF METHYL BROMIDE INJURY AS INFLUENCED BY MATURITY- AND TIME-ELAPSE BETWEEN PICKING AND FUMIGATION

Maturity	Time-elapse (weeks)	Methyl bromide injury index		
		All treatments	No. 7	No. 8
1	0	92.8	91.5	16.5
	2	30.9	12.0	16.0
	4	6.5	2.0	1.0
	6	11.2	0.0	0.0
2	0	26.9	2.5	0.5
	2	22.3	1.0	2.5
	4	12.3	6.5	0.0
	6	8.4	0.0	0.0
3	0	72.3	55.0	58.5
	2	19.3	3.5	3.0
	4	21.8	0.0	1.5
	6	3.8	0.0	0.0

From the figures in Table 6 it will be observed that if the apples are picked at the ideal stage of maturity (maturity 2) and are held in storage for two weeks, the extent of injury is reduced to insignificance by employing standard fumigation methods. Furthermore, by using these methods the extent of injury is completely reduced if a 6-week period is allowed to elapse regardless of maturity.

Relationship between Methyl Bromide and Injury to Apples:

From Table 7 it is obvious that there appears to exist very little correlation between the injury effects, as shown in the previous sections, and the amount of methyl bromide found by analysis. The illustration on Figure 1 shows that most of the injury is confined to the inner cortex and core area. Here again is found disagreement between analyses and the areas affected.

TABLE 7.—METHYL BROMIDE IN FUMIGATED APPLES

Treatment No. 7—Milligrams CH_3Br per kilo

Maturity of fruit	Time-elapse (weeks) between picking and fumigation	Core section	Inner cortex section	Outer cortex section	Whole apple	Condition of fruit
1*	0	11.0	14.0	19.0	18.0	5-internal injury.
1*	2	1.9	0.3	0.1	0.2	No injury.
1	4	1.5	0.3	0.2	0.2	1-internal; 1-skin lesion.
1	6	1.8	0.2	0.5	0.6	No injury.
2*	0	3.8	2.8	1.9	2.1	No injury.
2	2	4.4	0.6	0 7	0 8	No injury.
2	4	1.4	0.6	Nil	0.1	3-internal injury.
2	6	3.9	2.0	2.1	2.1	No injury.
3*	0	4 0	0.6	0.6	0.7	No injury.
3	2	Nil	0.3	<0.1	<0.1	No injury.
3	4	5.6	0.4	<0.1	0.3	1-internal injury.
3	6	10.5	0.9	1.9	2.2	No injury.

* Analysed by F. B. Johnston.
Untreated fruit gave no reaction for CH_3Br.

The only exception to these circumstances is found in the analyses of the first maturity sample fumigated immediately after picking. The high concentrations of methyl bromide in this case is difficult to explain.

Toxicity to Humans

The bromide residue in apples if sufficiently high would be toxic to human beings. Analyses were made to see if these residues were within a safe range of concentration in this respect.

Table 8 shows the results obtained on bromide residue determinations.

The method used would include any iodine present, but it is unlikely that this element is present in apples. Winton (17) gives a reference stating that apples do not contain iodine.

Neufeld (14) reports bromine in apples as 0.0003% on dry basis. Kitto (7) found less than one part per million on the wet basis. Damiens

and Blaignan (2) record the analysis of one sample of apples as containing traces of halogens.

The amounts of bromide residue found in fumigated apples do not appear to be large enough to be a factor in human nutrition. The increase in bromine content due to fumigation varied from 3 to 5 parts per million. If the bromine is fixed in the fruit tissue as inorganic sodium and potassium bromides, the amounts found are not physiologically significant, since it would require over 300 pounds of fumigated apples to furnish the average medicinal dose of these salts.

The Dow Chemical Company in the second edition of their booklet on methyl bromide state that "thorough analytical tests prove that the small amounts of bromine are present as inorganic bromides, such as potassium bromide, sodium bromide, etc."

TABLE 8.—BROMIDE RESIDUE IN FUMIGATED APPLES
Treatment No. 7—Bromine in mgms. per kilo

Maturity of fruit	Time elapse (weeks) between picking and fumigation	Total solids	As received	Dry basis	Increase in bromine due to fumigation		
					As received	Dry basis	Condition of fruit
1	0	13.31	6.1	45	5 2	39	5-severe injury.
1	2	11 85	4 5	38	3.7	32	1 slight injury.
1	4	12 40	4.1	33	3 3	27	No injury.
1	6	12.40	4 3	35	3.6	29	No injury.
2	0	13 33	4 5	34	3 7	28	No injury.
2	2	13 55	3 8	28	3 0	22	No injury.
2	4	13 18	4.9	37	4.1	31	No injury.
2	6	12.74	4 1	32	3 3	26	1—slight injury.
3	0	13 65	5.1	37	4.2	31	3—moderate injury.
3	2	13 44	4 6	34	3.7	28	No injury.
3	4	13.54	4.1	30	3 2	24	3—slight injury.
3	6	13.05	4.8	36	3 9	30	3—slight injury.
Controls		12.76	0.8	6	—	—	

Damiens and Blaignan (2) who have recorded the bromine content of a large number of fruits and vegetables report up to 26 milligrams of bromine per 100 grams dry matter (watermelons). Tomatoes were found to contain up to 5.3 milligrams of bromine per 100 grams of dry matter. These values are in excess of the amount found in the fumigated apples examined.

The amount of methyl bromide, as such found in the apples, is of a low order of magnitude, with the exception of 1st maturity sample treated immediately which appears to be abnormal. It is reasonable to assume that methyl bromide with a boiling point of 4.5° C. would soon escape from the fruit at higher temperatures.

DISCUSSION

From the experimental evidence it can be assumed that methyl bromide fumigation is practicable as applied for the destruction of insects infesting apples. The bromide residues resulting therefrom are well below

the danger point for human consumption.   However, there exists a grave problem in that as applied the methyl bromide fumigant under certain conditions results in both external and internal injury to the apples.

The injury is caused by methyl bromide itself since CO_2 and the vacuum treatment or combinations of each failed to produce the injury recorded.   The maturity of the apples as well as the delay between picking and fumigation had a profound influence on the resulting injury.   The addition of carbon dioxide in the vacuum fumigation treatment lessened the extent of the injury. Furthermore, no co-relation exists between the amount of methyl bromide found in the tissues and the injury involved.

All these factors point to the fact that the injury caused by methyl bromide to the apple tissue is due to a physiological upset rather than mechanical destruction.

Respiration curves established from continuous 24-hourly CO_2 output records of apples picked at various stages show an upset condition in the apples' metabolism shortly after picking (15).   This upset as shown by irregular and high rates is more severe in immature fruit.

If an apple is picked at the ideal stage the disturbance in CO_2 output at 55° F. lasts for about one week.   After this a sharp decline is noted lasting for an additional two weeks.   At this point a more uniform rate of CO_2 output exists.   There may possibly be some relationship between these changes in trend and the tolerance to methyl bromide.   If so it would seem that the apple is more able to resist the injury from the fumigant when a steady adjusted state of metabolism exists.

SUMMARY

The standard treatment of methyl bromide fumigation was found to be lethal to insects feeding internally in apples.   The residue of bromide was found to so slight as to be harmless to human beings.   Under certain conditions, however, methyl bromide under the various ways applied was found to cause both internal and external injury to the apples.   If the apples are picked at the proper stage of maturity and are stored for six weeks at 32° F. or 39° F. and standard treatment used no injury results. It is concluded the damage is physiological rather than mechanical.

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A Study of the Variation in Keeping Quality of Apples in Store : as Illustrated by the Behaviour of the Variety McIntosh Red from an Ontario Apple Orchard

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The storage of fruit with the object of lengthening the period during which it can be marketed is an aspect of horticultural practice which has become increasingly important in recent years. Where markets are close, it may be merely a question of extending the season of a particular fruit; but where, as now often happens, the fruit-growing district is a long way removed from its market, the problem of keeping the fruit in good condition during long journeys by rail or ship is added to that of delivering the fruit at a time when the market is ready for it.

The increasing need for efficient storage has brought a train of difficulties, the solution of which is the reason for much research work at the present day. The problems which need solution may be broadly divided into two classes : those of the engineer and those of the horticulturist. The latter class, with which the writer is immediately concerned, may again be subdivided. Thus it may be the object of research to find the particular conditions of temperature or atmosphere in which a given fruit keeps best, or it may be necessary to adapt the culture of the fruit to the known limitations of the store. In either case it is necessary to examine the storage life of fruit grown in varying circumstances under a range of conditions in store.

There are many ways in which the life of a fruit in store may end from the commercial point of view, and one of the first points to decide in planning an experiment in keeping quality is the criterion by which the results are to be judged. In some experiments of a commercial nature it is sufficient to note that the fruit is sound or the reverse—the cause is immaterial; but it is more often necessary to analyse more carefully the immediate causes of breakdown and to estimate their extent under different sets of conditions. This is often a considerable problem in itself. Thus the breakdown may be due to senescence uninfluenced by other causes, or fungal invasion may cause the rotting of the fruit. In

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the case of apples in cold store, such disorders as core-flush or external scald may be a direct result of the temperature at which the fruit is stored. Where the fruit is kept in an artificial atmosphere, there are certain disorders which are the result of incorrect gas mixtures for the type of fruit stored.

The length of life is by no means the only information required in storage experiments. Such qualities as firmness, appearance and flavour are also of great importance, and are even more difficult to measure.

One of the main difficulties has been to decide upon an adequate sampling method in the selection of fruit for storage. It is evidently impossible to store all the fruit of whole trees in order to compare different treatments; indeed, if the treatments are concerned with the life of the fruit in store, it is desirable to use samples of fruit from the same trees for each variation in storage condition. The worker is therefore forced to select a number of samples of fruit from each tree. Just how best to select a sample, and what size of sample will give the greatest efficiency, are matters which have not yet been agreed upon by research workers, though all are agreed that samples of fruits, even from a single tree, are generally very variable. Some of the causes of this variability have already been established. Thus maturity,[4, 17] size,[14, 16, 17, 21] colour,[11, 17, 18] and even position on the tree [8] have all been shown by various workers to influence one or another storage quality, whilst such factors as the age of the tree [21, 23] and the relative heaviness of the crop have also been shown to be important.[2, 17]

To meet different contingencies individual workers have developed their own methods of sampling. Thus in studying the effect of varying atmospheres and temperatures on apples in gas storage, some have been at pains to spread the variability over all samples.[5, 23] Thus, if the crops of a number of similar trees are available, samples of, say, 100 apples are decided upon, and each sample receives an equal number of apples from each tree. In order to reduce the variability, only apples of medium size are used. This method has an evident flaw, where fruit from, say, differently manured plots is being compared. For while the direct effect of manuring on keeping quality, if any, may be shown, the indirect effect whereby manuring influences, say, colour, size or maturity, and thus keeping quality, may be masked.

Accordingly, other workers have selected samples containing only typical fruit of the variety or treatment being sampled.[21] The success of this method must depend on the experience and skill of the harvester, and the sampling cannot be easily delegated by him. Further, there is always the danger of bias, even by the most

experienced workers. It seems, therefore, that if methods of sampling could be evolved which could be easily carried out, and which would yield data from which conclusions of statistical significance could validly be drawn, it would be well worth while. The present paper describes an attempt to do this for Canadian conditions in the winter 1936–37 by the writer in collaboration with members of the Division of Horticulture during a period of residence at the Central Experimental Farm, Ottawa.

MATERIAL

The experiment was carried out on McIntosh Red apples obtained from a single orchard owned by Miss Tindale, and situated at Iroquois, Ontario, on the banks of the River St. Lawrence, about 60 miles south of Ottawa. The fruit, when harvested, was transported to Ottawa, where it was stored in air in two experimental chambers, differing only in the temperatures maintained therein. The particular orchard was, in this season, practically the only one within reasonable distance of the Central Experimental Farm which was at all suitable. There were two reasons. In the first place, spring frosts in 1936 had caused a considerable reduction in crops, and one or two possible alternatives had to be discarded on this account. Secondly, the fruit-growing districts of Ontario were in 1936 still suffering from the disastrous winter of 1933–34, when, owing to an early freeze followed by a very severe winter, widespread winter injury to the trunks and branches of all kinds of fruit trees was experienced. The result was that there was hardly a mature orchard to be found in this part of Canada which was wholly unaffected. The orchard at Iroquois finally selected had suffered like the rest, but perhaps not so severely as some. Nevertheless, while it was possible to find but few uninjured trees, there were sufficient only slightly damaged trees to make the experiment worth while. In addition, nearly all were carrying heavy crops. As will be seen, a comparison of the effect of different degrees of winter injury upon keeping quality was included in the experimental design, since it seemed probable that in this neighbourhood storage research workers would have to rely for their material, at least for several years, on trees which have suffered to a greater or lesser extent. It seemed therefore desirable to know how far the produce of such trees could safely be used.

In Canada it is usual to grow rather larger trees than in England, and thus the produce of a single tree is often comparatively large. Some of the trees in this orchard yielded well over 20 bushels (800 lb.) each in the season of the trial. With crops of this size it was possible to obtain quite a large number of small samples from each tree, and,

further, to get accurate information as to the relative qualities of different kinds of apples from the same tree.

Uniformity trials may be of two kinds. The more usual method is to set a minimum-sized sample and to divide the material up into units of this size, each of which is harvested and recorded separately. These samples, when combined, may make up the whole of the available material, as for instance when a field of wheat is divided up into a number of small plots and the whole harvested as individual units. If the whole population is too large to be treated in this way, it is usual to draw samples by some process of random selection. In either event the unit samples are then combined in various ways to form larger samples, from which the most efficient size for experimental purposes is eventually deduced.

A more elaborate form of uniformity trial, such as was done at East Malling on strawberries, is to design the experiment so that some of the possible causes of variability in the so-called uniform material may be separated out and their effects estimated. The elimination of these disturbing factors in the analysis of the results gives an estimate of the reduction in variability which may thus be accomplished. In such trials the samples are not selected wholly at random from the whole. The available material is graded into classes for various characters, and an equal number of samples is selected at random from each class. It will be noted that as the population is not likely to be equally divided up by this grouping, the samples selected will not represent the whole population. But the samples from each class will be representative of the group from which they are taken.

In horticultural work this kind of uniformity trial is of particular value, since normally most of the material is comparatively variable, and research workers are constantly concerned to find methods of increasing its uniformity.

In the present investigation both kinds of trial were attempted, one series of random samples being obtained, and a second series, designed to throw light on possible causes of variability both before and after placing in store.

THE EXPERIMENTAL DESIGN

1. *Selection of Trees*

A careful tree-to-tree survey of the orchard was first undertaken about a fortnight before harvest, and upon this basis a detailed scheme was drawn up. This scheme had subsequently to be somewhat modified when picking commenced, but the main lines of it were unchanged. At this survey the orchard was mapped and visual records were made for each tree of the amount of winter

injury and the relative crops borne. For winter injury the trees were divided into six categories, designated o to 5, according to the amount the tree had suffered. Trees which had no visible injury were recorded as o, and the figure 5 implied that the whole tree was affected.

In recording the crops no attempt was made to estimate the actual yields, but the trees were recorded as bearing light, medium or heavy crops for their size. It was considered that this was the more important, as it had already been shown that fruit from trees in their " off " season kept less well than that from trees bearing a heavy crop.[2, 17]

Records of disease, such as scab or fire blight, were also obtained.

On the basis of this survey 32 trees were selected for the trial, the following factors determining their choice :--

> (*a*) Amount of winter injury;
> (*b*) Freedom from disease;
> (*c*) Size of crop;
> (*d*) Position in the orchard.

(a) *Winter Injury*

Trees from three of the above-mentioned categories were used.

Group (i), Categories o and 1. It was not possible to find sufficient trees with no injury at all, and this group included the few uninjured trees and those which had slight trunk injury the effect of which was not apparent in the branches.

Group (ii), Category 2. These trees had some trunk injury, and one or two small branches dead or poor leaf on one limb. Fruit from the affected limb was not used.

(b) *Freedom from Disease*

Trees showing excessive amounts of scab or fire blight were discarded and, just before harvesting, three selected trees had to be replaced as some of the apples proved to be suffering from internal cork. This disorder is believed to be associated with a deficiency of boron and, unless severe, the symptoms are not externally visible.

(c) *Size of Crop*

Only trees bearing more than average crops were used, with three exceptions, where the crop was only average.

(d) The orchard was divided as far as the distribution of suitable trees would allow into four blocks each containing 8 trees, 4 of each of the two groups for winter injury being included in each block. In practice this was difficult, since the available trees were not

distributed evenly over the ground. But for what they were worth, the 4 blocks were kept separate and labelled according to their position N.E., N.W., S.E. and S.W. Fig. 1 shows a plan of

FIG. 1.—Plan of Miss Tindale's Orchard at Iroquois, Ontario, showing position, winter injury and crop of trees in the trial.

0 = No apparent injury.
1 = Slight trunk injury.
2 = Trunk and slight branch injury.

32 = Stored in room kept at a temperature of 32° F.
36 = Stored in room kept at a temperature of 36° F.

M = Medium crop.   MH = Medium to heavy crop.   H = Heavy crop.

the orchard in which the positions of the selected trees and the 4 orchard blocks are marked.

2. *Method of Sampling*

Six samples, each of 60 apples, were selected from each tree. The size of sample chosen was largely a matter of convenience, 60 fruits occupying each of the storage-trays in which the fruit was placed. These samples were of two kinds. Two were obtained by a process of random selection direct from the trees, while each of the other 4 consisted of 12 sub-samples of 5 apples, each sub-sample representing a certain type of fruit.

(a) *Random Samples*

The method of sampling was to approach the tree with the hand outstretched and eyes shut until a twig was touched. The branch to which the twig belonged was followed up to its highest point. Starting at this point, all the fruit was picked, working down the branch, until the necessary 60 apples were obtained. The process was then repeated at approximately the opposite side of the tree, avoiding winter-injured branches. All samples had a few extra apples to cover the risk of damage in transport.

(b) *Graded Samples*

The graded samples were obtained in a somewhat different manner.

In the scheme it was desired that the 12 sub-samples should represent differences in the fruit within a tree which might influence the storage life.   Three factors were originally included :—

     (*a*) Size.
     (*b*) Maturity.
     (*c*) Position on the tree.

Of these (*a*) was the only factor which was eventually included as originally conceived.   Maturity has been considered to be one of the principal factors influencing storage life.[4, 17]   In an apple it may be estimated in three ways : (i) the ground-colour of the fruit, (ii) the reaction to the iodine test for starch and (iii) hardness.   Of these only the first can be observed without damaging the fruit.   It was found on examination that at the same date it was very difficult to detect any difference in ground-colour throughout the orchard, and quite impossible within one tree, so that this factor was abandoned and replaced by the amount of red colouring of the fruit.   With regard to position on the tree, it was intended to divide the crop into " king apples "—*i.e.*, apples from the centre blossom of a truss—and apples from side flowers, since there was evidence to show that these " king apples " were physiologically different, and they were generally reputed to keep badly.   However, the majority of the trees bore for the most part single fruits to a truss, and at this stage it was not possible to determine from which kind of flower they came.   This factor also was therefore discarded, and replaced by a comparison of apples from terminal fruit-buds—*i.e.*, buds at the tip of a one-year-old shoot—and those from spurs —*i.e.*, short side-growths two or more years old.   The three factors finally chosen were thus (i) size, (ii) red colouring, (iii) position on tree, and the method of grading was as follows :—

(i) *Picking.*—After selecting the random samples, the remaining fruits were harvested, spur and terminal apples being kept separate.   It was found that from 4 to 5 bushels of each from every tree were required to obtain therefrom the necessary number of fruits in each size and colour category.   This meant that to obtain the 240 apples needed for four samples of 60 apples each, from 800 to 1000 fruits had to be graded for each tree.   The necessary amount was taken at random from the whole crop; this as a rule included all the terminal fruits.

(ii) *Size Grading.*—The next step was to grade for size.   This was done by hand through a ring grader, the fruit being carefully

handled to avoid bruising. The difference between the grades was a quarter of an inch in the diameter of the ring. Three sizes were retained :

Grade 1 ... ... 2¼ to 2½ ins.
Grade 2 ... ... 2 to 2¼ ins.
Grade 3 ... ... 1¾ to 2 ins.

Grade 1 included those apples which rested on the 2¼-inch ring, but passed through the 2½-inch ring, and similarly for grades 2 and 3. Outsize and very small fruit were discarded. Damaged fruit, apples picked without their stalks and badly scabbed fruit were not used.

(iii) *Colour-grading.*—The fruit in the three selected size grades was next subdivided into three further grades for amount of the surface coloured red. These were :—

(a) No red colour and up to and including one-third of the surface coloured.

(b) Over one-third coloured and up to and including two-thirds coloured.

(c) Over two-thirds coloured.

This grading was done by comparison with two standards, each consisting of a few fruits at the top of grades a and b. Any fruit having more colour than the first standard but not more than the second went into the middle grade. In actual practice a certain amount of latitude had to be allowed in this colour-grading, owing to differences in intensity and the occurrence of striped apples. Errors of judgment were minimized by allowing one man to do the whole of the colour-grading.

Of the three grades, only grades a and c were retained. The rejection of grade b meant that a very large proportion of the fruit was not used, and this accounted in a large measure for the extra fruit which had to be picked. It was desired, however, to get a clear line between the two colour categories. From the fruit which remained after the several gradings, four samples each of 5 apples from each of the 12 categories were selected at random. Where possible an extra apple was included in case of damage in transport.

It may be noted that individual trees varied considerably in red colouring, so that it was not possible to obtain identical samples from all trees. The chief differences between the samples finally chosen from different trees were, however, in intensity rather than in the amount of surface area coloured. None the less it is probable that all samples from some trees came towards the top of the class interval, while in others the whole batch would tend towards the bottom of the class interval. In view of the great influence of

colour on keeping quality subsequently shown, this may have accounted for some of the differences between trees.

In a few cases, especially in size grade iii, it was difficult to obtain sufficient terminal apples to make up the required sub-samples. The 12 categories into which each sample of 60 fruits was divided were as follows :—

1.	Terminals ...	...	...	Size i.—Red	
2.	,,	...	...	...	,, — Green
3.	,,	...	...	...	Size ii.—Red
4.	,,	...	...	...	,, —Green
5.	,,	...	...	...	Size iii.— Red
6.	,,	...	...	...	,, —Green
7.	Spurs	...	...	...	Size i.—Red
8.	,,	...	...	...	,, — Green
9.	,,	...	...	...	Size ii.—Red
10.	,,	...	...	...	,, —Green
11.	,,	...	...	...	Size iii.—Red
12.	,,	...	...	...	,, —Green

Arrangement of the Fruit in Store

The fruit was stored in air in two cold-storage chambers at the Central Experimental Farm, Ottawa. In one the temperature was maintained at 32 F. until the middle of May, and in the other the temperature was 36° F. The latter was considered from previous experience to be about the ideal temperature for McIntosh Red,[4] but, as it was by no means proved that this would be constant under different seasonal conditions, it was decided to include a second temperature. 32° F. was chosen, as it was considered probable that certain disorders connected with low-temperature storage might develop at this temperature, whereas at the higher temperature they might not occur. All the samples from 16 trees were placed in one room, and those from the other 16 trees in the second. These included 4 trees from each orchard block, 2 in Category 1 for winter injury and 2 in Category 2. The room in which the produce of each tree was stored is shown on the plan (Fig. 1), being indicated by the number 32 or 36.

The arrangement of the graded samples within the two rooms may be compared with that of a field experiment in four random-ized blocks with split plots. In this type of experiment certain treatments, as a rule likely to show broad differences, are arranged in the usual way in randomized blocks, rather large plots being used. Each plot is then subdivided into a number of smaller plots, to each of which is applied one of a second series of treatments.[24] As far as the writer is aware, no attempt has previously been made

to estimate variations in keeping quality which might be associated with differences in position within the storage-chamber, though it has been shown that quite small differences in temperature in a large store may influence the amount of breakdown.[13] In these comparatively small chambers precautions were taken to ensure that the temperature and humidity throughout the room were kept as constant as possible, and it was not considered likely that large differences would occur. Nevertheless it was determined to include the possibility in the experimental design. Each room was therefore divided into four blocks, each block consisting of 16 storage trays in eight layers of two trays each. Each tray contained a single sample of 60 apples from each of the 16 trees represented in the room, its position within the block being determined by chance.

Each tray was divided into 12 compartments, each to contain a sub-sample of 5 apples corresponding to the 12 categories listed above. The position of each sub-sample within the tray was also predetermined by a process of random selection.

This "split plot" design was more or less dictated by the circumstances of the experiment, since it would have been humanly impossible to randomize completely all the 192 sub-samples in each room without error and delay, some of them being harvested nearly a week later than others. As it was, an elaborate organization was necessary to ensure that all the fruit was in store within 24 hours of picking.

The 32 random samples, each of which also occupied a single tray, from the two sets of 16 trees were placed above the graded samples in each room; the actual position which each occupied was determined entirely by chance, thus imitating the more normal method of arrangement of experimental material in store.

When all these trays were stored, there remained room for some 24 trays in each room. Part of this space was filled with a series of extra samples, selected entirely at random from similar fruit not already used, which were subsequently removed at fortnightly intervals to watch the progress in store.

Organization of the Harvest

The magnitude of the experiment necessitated careful organization. The actual picking and grading took 5 days, the second day being lost owing to heavy rain. During this time extreme variations in climate were experienced, as is shown by the weather records at Ottawa during the period (Table I).

While these records are not strictly applicable, they give a reasonably good picture of the variations at Iroquois during the week.

The fruit was considered to be rather under mature when picking commenced, but it is probable that some ripening took place before all the trees were picked. The effect of this within a room was minimized by filling the whole of room 32° F. in the first two

TABLE I

Weather Records at Ottawa, September 23rd–28th, 1936

Date				Temperature		Rainfall	Sunshine
				Maximum	Minimum		
Sept. 23	...	...	...	84	55	0·28	8·7 hrs.
,, 24	...	...	...	67	47	0·78	none
,, 25	...	...	...	49	35	none	8·7 hrs.
,, 26	...	...	...	64	32	,,	7·6 ,,
,, 27	...	...	...	61	49	1·00	8·7 ,,

days and room 36° in the last. It was not possible to confound the time difference with the orchard blocks in any way, as this would have meant constantly shifting the trays during the harvesting period.

As soon as the fruit was picked it was graded on the spot. Four men did the picking, two size-graded, one colour-graded, and one man packed. When graded, the samples were packed directly into the trays in which they were to be stored, each sub-sample in its appropriate compartment. They were then transported by road to Ottawa, where they were unpacked, examined, re-wrapped in oil-impregnated wrappers and placed in store. Expedition between picking and storing being known to be of importance,[1, 14, 16] all the fruit was placed in store within 24 hours of picking.

Recording the Results

With the coming of the New Year a start was made in the examination of the extra samples included for this purpose. The reason for this periodical examination was the determination of the best time at which to remove all the fruit of one or both rooms. This date had to be carefully arranged, as it was evidently futile to examine the fruit either too soon, when there might be no evidence of breakdown, or too late, when all the fruit might have gone too far. It was decided that when about 25 per cent. of the sample was affected it would be about time to remove the whole. The examination would actually take place a week later, since after the removal all fruit would be kept in a ripening-room at a constant temperature (65° F.) and humidity (85–95) for a week.

It was soon evident that, in spite of under maturity at harvest, this fruit was going to keep exceptionally well. And by the third

week in May 1937, although a certain amount of shrivelling and fungal rotting had taken place, there was very little evidence of breakdown. (This was unfortunate from the writer's point of view, as he was unable to be present when the whole was eventually removed.) The reasons for this good keeping were probably connected with the careful handling of the fruit, expedition in storing and favourable storage conditions. Nevertheless at this date it was determined that the fruit could not be kept in store much longer, and accordingly an attempt was made to induce breakdown by varying the temperatures of the store-rooms. This is easy to justify, since the experiment was concerned only with comparison of certain kinds of fruit under a given set of storage conditions. The actual conditions were immaterial, since the ideal storage temperatures were not in question.

The temperature of the 32° F. room was therefore lowered to 30·5° F. on May 22nd, and kept at this level for approximately 10 days, when it was raised to its original level.

The temperature of the 36° F. room was at the same time lowered to 34° F., and kept there for a similar period. The process was repeated in this room, but for a shorter period of 3 days, on June 5th.

It should be noted that, although up to this time there was very little breakdown in either room, from the market point of view the fruit had been kept far too long. That in room 36° F. was decidedly over-ripe, with a certain amount of shrinkage, and flavour and texture were both well past their prime. The apples in room 32° F. were not so far advanced, but none the less definitely over-ripe.

The device proved effective, and the fruit was ready for removal from the 36° F. room on June 17th, and from the 32° F. room on June 21st.

On removal, after the necessary interval in the ripening-room, the examination of the fruit took place. Each apple was examined and recorded separately. After fungal rotting and superficial scald had been recorded, the fruit was cut and the amount of core-flush estimated. Records of amount of shrivelling, evidence of disease and flavour were also made for the whole sub-sample of 5 apples.

The most important of these records from the point of view of this experiment were those of superficial scald and core-flush. Both are forms of breakdown connected with low-temperature storage. The former takes the form of a browning of the skin of the fruit, though the interior may be unaffected. The latter is a form of internal breakdown which is characterized by a discoloration of the flesh in the neighbourhood of the core. Both conditions are capable to some extent of numerical estimation, and this was done in much

·the same way as the colour grading before storage. Each apple was designated o, 1, 2 or 3, according, in the case of scald, to the relative amount of surface area browned, and, in that of core-flush, to the proportion of the core area affected and the depth of colour, estimated by eye. Standards were used for comparison at the top of categories 1 and 2 in the same way as in the colour-grading.

For each sub-sample of 5 apples there were thus records of scald and core-flush which could vary from o to 15 according to the severity of breakdown of each of the fruits composing it.

In some cases, particularly in the 36° F. room, fungal rotting involved all or most of a fruit, and this could not be included. In the subsequent analysis of these records the figures for sub-samples containing less than 5 apples were adjusted accordingly.

In samples of 60 apples the records could vary from o to 180. Table II shows the relative amounts of scald and core-flush per

TABLE II

Average Amounts of Scald and Core-Flush in the Two Rooms

Mean per sample of 60 apples

	Room 36°		Room 32°	
	Scald	Core-Flush	Scald	Core-Flush
Random samples	29·2	23·8	91·6	86·9
Graded samples	40·8	28·4	78·7	62·6

sample of 60 apples in the two rooms. As might be expected, both forms of breakdown were much farther advanced in the room with the lower temperature. It is difficult to decide just why the random samples show a higher difference than the graded samples. While the results would not be expected to be the same, since the one is representative of the whole crop and the other of only part, it is not clear how the apparently different result in the two rooms came about.

These differences include those both in number of apples affected and in the degree. In the random samples, for example, room 36° F. had about 32 per cent. of the fruit scalded, with an average intensity of 1·5 (*i.e.*, if the centre of the class interval is 1, at about the limit of category 1). In room 32° F., 71 per cent. of the fruit had scald with an intensity of 2·1 (rather above the centre of category 2). It will be seen that while room 36° F. had reached about the point at which it was originally intended to remove the fruit, room 32° F. had gone very much further.

Analysis of the Results

(a) *Random Samples*

The first step was to analyse the data for the two sets of 32 random samples. The two rooms were treated separately throughout, for reasons which will be understood later, when the big difference in variability between the two rooms is seen. The method

TABLE III

Amount of Core-Flush in Random Samples of 60 Apples each in Room 36° F.

Orchard Block	S.E.		S.W.		N.E.		N.W.		Totals
	Row, Tree and Sample		Row, Tree and Sample		Row, Tree and Sampe		Row, Tree and Sample		
Winter injury 1	9, 14a	20	11, 6a	25	2, 8a	33	8, 4a	30	
	9, 14b	26	11, 6b	3	2, 8b	6	8, 4b	25	
	9, 15a	21	12, 6a	21	5, 15a	18	8, 5a	14	
	9, 15b	12	12, 6b	33	5, 15b	47	8, 5b	11	
Two trees total	—	79	—	82	—	104	—	80	345
Winter injury 2	6, 11a	46	11, 5a	6	2, 9a	33	7, 6a	4	
	6, 11b	65	11, 5b	2	2, 9b	9	7, 6b	7	
	9, 13a	8	13, 1a	56	4, 1a	76	9, 5a	3	
	9, 13b	26	13, 1b	41	4, 1b	18	9, 5b	15	
Two trees total	—	115	—	108	—	136	—	29	418
Four trees total	—	224	—	190	—	240	—	109	763

used was the analysis of variance [6] and the process is illustrated from the records of core-flush in room 36° F. Table III shows the actual amount of core-flush for each random sample of 60 apples in this room. The 32 samples, it will be remembered, were obtained by taking two from each of 16 trees. Four of the 16 trees were situated in each orchard block, 2 in each category for winter injury. The first analysis on single samples is as follows :—

TABLE IV

Analysis of Variance : Samples of 60 Apples. Core-Flush. Room 36° F.

Variance due to :	Degrees of Freedom	Sum of Squared Deviations	Mean Square (σ^2)	$\frac{1}{2} \log_e \sigma^2$	S.D.
Between trees ...	15	7,250·7	483·4	3·0904	21·99
Within trees ...	16	3,643·5	227·7	2·7141	15·09
Total	31	10,894·2	351·4	—	18·75

The total variance for single samples from all trees is shown in the last line of the Table, and is 351·4. This gives a standard

deviation of 18·75. The general mean per sample is 23·84, so that the coefficient of variability is 78·6 per cent., a very high figure. This total variance may be split up into two parts : that due to differences between trees, which is 483·4, and that between samples from the same tree, which is 227·7. Comparing these two by the " Z " test,* a value of $Z = 0.3763$ is obtained. For $n_1 = 15$ and $n_2 = 16$ the 5 per cent. point for Z is found to be about 0·44, so that the difference is not significant. Nevertheless by eliminating differences between trees a reduction in the standard deviation is obtained to 15·09 or 63·3 per cent. of the mean. This represents the variability of samples taken from the same tree. From this table also can be obtained an estimate of the variability of single samples taken from different trees. If σ_w^2 be taken to be the true variance of single samples from the same tree, *i.e.*, that just examined, and σ_r^2 be the true variance between the means per sample of different trees, then the variance of single samples taken from different trees will be $(\sigma_r^2 + \sigma_w^2)$. The first line of Table IV, calculated from the totals of two samples per tree, gives an estimate of $(2\sigma_r^2 + \sigma_w^2)$, while the second line gives an estimate of σ_w^2, whence can be calculated $(\sigma_r^2 + \sigma_w^2) = 355.5$. The square root of this is the standard deviation of single samples from different trees, which is 18·85 or 79·2%. While the significance of the differences between these very high values of the coefficient of variability is in doubt, it does appear likely that samples from the same tree will be more like one another than those from different trees, and this is confirmed by the other records.

The variance between trees can be further split up to show the effect of position and of winter injury as in the further analysis in Table V.

It is evident in Table V that there were no significant differences connected with either position or with amount of winter injury, and the elimination of their effect has been to increase rather than decrease the standard deviation. Further, there is no difference between the two components of error—*i.e.*, between and within pairs of trees in the same block. This might be expected from the scattered distribution of trees composing each like pair within each block.

Table VI gives the coefficients of variability obtained for both core-flush and scald in each of the two rooms. It will be noted at

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* The " Z " test, originally described by Fisher,[6] is the usual method of comparing two values of the variance. " Z " is equal to half the difference between the natural logarithms of the two variances. Tables of " Z " for two levels of significance P = 0·01 and P = 0·05 have been given by Fisher for different values of n_1 and n_2 the degrees of freedom appropriate to the larger and smaller variances respectively.

once that with the higher amounts of both forms of breakdown in room 32° F. a considerably greater uniformity is obtained. This is to

TABLE V

Analysis of Variance between Trees. Core-Flush. Room 36° F.

Variance due to :	D.F.	Sum of Squares	Mean Square	$\frac{1}{2}\log_e$	S.D.
Position	3	1,276·8	425·6	—	—
Injury	1	166·5	166·5	—	—
Error (between pairs of trees)	3	915·6	305·2	2·8605	—
Error (within pair of trees)	8	4,891·7	611·5	3·2079	—
Both	11	5,807·3	527·9	—	22·90
Total	15	7,250·7	483·4	—	21·99

TABLE VI

Coefficients of Variability for Core-Flush and Scald obtained for Random Samples of 60 Apples in each Room

	Room 36° F.		Room 32° F.	
	Core-Flush	Scald	Core-Flush	Scald
Mean amount per sample	23·8	29·2	91·6	86·9
Coefficient of variability.				
Between trees	79·2	77·0	34·5	47·7
Between samples within trees ...	63·3	37·8	29·4	47·8
Total	78·6	75·2	34·3	47·7

TABLE VII

Effect of Increasing the Size of Random Samples

	Room 36° F.		Room 32° F.	
	Core-Flush	Scald	Core-Flush	Scald
1 Sample from 1 tree	79·2	77·0	34·5	47·7
2 Samples from 1 tree	56·0	54·5	24·4	33·7
1 sample from each of 2 trees ...	54·4	89·7	18·2	31·2
2 Samples from 2 trees	38·5	63·5	12·8	22·0

be expected from the nature of the data, which involve both the number of apples affected and the degree. This point will be further discussed later.

The variability of the whole 32 samples is much the same for core-flush and scald in the same room, but there appears to be a greater difference between that between trees and that within trees in the 36° F. room. This is especially so for scald, where fruits from the same tree are significantly more uniform than those from different trees.

From the practical point of view of sampling plots of trees in a manurial experiment, for example, it may be asked what is the effect of increasing the sample size. This is shown in Table VII. Should the plots consist of individual trees, 2 samples from each tree give a coefficient of variability equal to that for 1 sample divided by $\sqrt{2}$. Where there are 2 or more trees in a plot, the effect of taking 1 sample from each of 2 trees, as compared to a double sample from 1 tree, will depend largely upon whether pairs of trees within a block are more alike than pairs of trees far apart. If they tend to be alike. there is no advantage in spreading the sample over 2 trees. The scald records show an example of this, pairs of trees within blocks tending to vary much less than pairs of trees far apart. Where pairs of trees within blocks are not more alike than those farther apart, there is a distinct advantage in sampling from 2 trees rather than from a single tree. The core-flush records are an instance of this. Doubling the size of the sample again and taking 4 samples, 2 from each tree, further reduces the variability. Just why there is more advantage in taking 2 trees for core-flush than for scald is not clear; but some light may be shed on this fact in the subsequent section, when the reasons for the variability within samples are discussed. Pairs of trees may have differed much in the average size of their fruit. Fruit-size is shown later to influence core-flush, but not scald.

It seems clear, at any rate, that unless very large samples are used—and even then unless the amount of breakdown is fairly high– random samples are likely to be very variable. It remains, therefore, to discover the source of this high variability and try to eliminate it, either by a different method of sampling, or in the statistical reduction of the results.

(b) *Graded Samples*

It will be remembered that the graded samples came from the same trees as the random samples, but that there were 4 samples from each tree instead of 2. Each of these 4 samples occupied a random position in one of the four blocks in each room, and consisted of 12 sub-samples in one tray, each of 5 apples, representing the various grades of colour, size and position on the tree.

The analysis of the records on these samples is thus analogous

to an experiment in four randomized blocks each containing 16 plots (1 sample of 60 apples from each of 16 trees), each plot being split into 12 sub-plots (1 sub-sample of 5 apples from each of the 12 kinds of fruit from the same tree).

The analysis of this kind of experiment is carried through in two stages (24), Part I being the analysis of whole plots, from which an estimate of error applicable to the comparisons of whole plots is obtained, and Part II being the further analysis of the " split plots," from which a second estimate of error is obtained

The records of this experiment have been treated in exactly the same way, and to illustrate the method and results, the analysis of the records of amount of core-flush in the two rooms will here be set out in detail

Analysis of Core-Flush Records in Both Rooms

Part I   Whole Samples of 60 Apples.

Table VIII summarizes the differences between whole samples associated with position in the orchard, winter injury and position in the storage-chamber, for both rooms   Each figure in the Table

TABLE VIII

Amount of Core-Flush per Sample of 60 Apples in Each Room

Room Block		Room 36° 1					Room 3_ 1				
Or chard Block	In jury	I	II	III	IV	Means	I	II	III	IV	Means
B L	1	16 9	17 1	26 0	_4 5	_1 1 } _1 6	5 5	(1 C	C (	71 0	6_ 7 } (1 0
	2	1 3 0	31 0	29 5	83 _	_8 _	C 0 3	5 3 3	58 0	5 3 5	5 3 3
B W	1	25 C	__ 1	38 5	37 1	30 3 } 1 7	50 (	(4 5	(1 5	8 0	(1 8 } (6 3
	2	_4 0	_7 _	31 9	(6	32 4	71 0	49 7	75 0	80 1	(8 3
N L	1	37 7	37 3	35 (	43 5	38 5 } 3_ 7	5 3 7	55 5	59 3	53 7	57 1 } (5 0
	2	3 1	15 5	_3 3	3 3 4	_(9	61 3	6 3 3	73 0	81 5	7_ 5
N W	1	17 8	_5 3	1 0	_0 0	_1 8 } _1 5	6 3	(8 0	C_ 2	(5 5	(5 _ } 57 6
	2	20 1	_7 0	__ 8	36 (	_7 1	54 3	38 0	5 3 8	5 3 8	50 0
Mean		_4 3	_((	_3 0	(	Gen mean _8 37	6 1	57 3	(3 C	(8 3	Gen mean 6_ 60

represents the mean per sample of 60 apples   In the body of the Table each is the mean of 2 samples, 1 from each of 2 similar trees Columns 5 and 6, and 11 and 12 show the effect of winter injury and orchard position, while at the foot of each half of the Table the differences between the four parts of the storage-chamber are summarized

These data are analysed in Table IX   As in the random samples there are 15 degrees of freedom for differences between trees, divided

up into the parts due to position in the orchard, injury, the inter-
action of the two, and differences between pairs of trees in the same
block. In addition, there are now 3 degrees of freedom for differ-
ences in position within rooms, which leaves 45 out of the whole
63 degrees of freedom for error. The sums of squared deviations are

TABLE IX

Analysis of Variance, Part I, Samples of 60 Apples.   *Core-Flush*

Variance due to	Degrees of Freedom	Room 36° F.			Room 32° F.		
		Vari-ance	" Z "	S.D.	Vari-ance	" Z "	S.D.
Room blocks	3	254·09	**0·6871**	—	381 27	**0·6464**	—
Trees.							
Orchard blocks	3	312·27	**0 7902**	—	275·35	0·4837	—
Injury	1	5 12	—	—	1·82	—	—
Interaction of orchard blocks and injury ...	3	287 61	**0 7491**	- -	675·36	**0·9323**	---
Between trees	8	671·89	**1 1734**	- -	826·04	**1 0330**	- -
Between samples (error)...	45	64·29	—	8·019	104·65	- -	10·230
Total	63	171 99	—	13 114	243·10	- -	15 592

omitted for the sake of brevity, and the variances appropriate to the
different component parts are listed in columns 2 and 5 for each of
the two rooms. Each of these estimates of variance has been
compared with that due to error, and the values of Z obtained.
Significant values of Z are printed in block type.

Examining these analyses, it is clear that the results are alike
in both rooms. Firstly there are significant differences due to
position within the rooms. These are not large, the value of *P*
being less than 0·05, but more than 0·01. Nevertheless examina-
tion of Table VIII shows a distinct difference in favour of blocks I
and II. There is no apparent reason in connection with the layout
of the rooms why blocks III and IV should have more core-flush;
and the explanation probably lies in the fact that in examining the
fruit from both rooms, blocks III and IV were dealt with a day
later than blocks I and II, and were in consequence a day longer in
store, though the same period in the ripening-room. Core-flush
must have been increasing rapidly at the time, and this possibly
accounts for the difference. If this is so, it is evidently important,
when a large quantity of fruit has to be recorded, to spread samples
from all treatments over the whole period of examination.

Orchard blocks show significant differences in room 36° F., but
not in room 32° F., though the same blocks, N.E. and S.W., show
the largest amount in both rooms. Winter injury has had no
definite effect in either room, but appears to have been associated
with a greater amount of core-flush in some blocks and with less in
others.

This curious result may be partly accounted for by the difficulty of making an exact estimate of amount of winter injury and the rather small difference between categories 1 and 2. On the other hand, it is more likely to be due to chance; usually it is a single tree which is responsible for a marked deviation one way or the other. When the marked effects of size and colour, to be shown in the second part of the analysis, come to be considered, and it is remembered that some categories were difficult to fill adequately, possible explanations suggest themselves. For example in room 36° F. one of the trees in block N.E., winter injury 1, which showed much more core-flush than the other, was noted as having exceptionally large fruits.

When the above factors have been separated out, there are still marked differences within like pairs of trees. Various factors may be responsible for this. There were considerable variations in fertility within the orchard, notably with respect to nitrogen, and these were not by any means accounted for by the positions of the orchard blocks, dictated by the few suitable trees available. Some of these variations may have had an indirect effect on core-flush through size or colour of the fruit, or there may have been a direct effect. In any case, it is clear that with these " uniform " samples, differences between whole trees, even when they were close together, were much greater than those within trees.

It is interesting to compare the standard deviations obtained for whole graded samples with the random samples. In room 36° F. the graded samples gave a standard deviation between samples of 8·019 or 28·3 per cent., as compared with 63·3 per cent. for random samples of the same size. In room 32° F. the standard deviation was 10·23 or 16·3 per cent. as compared with 29·4 per cent. in the random samples (see Table VI).

Part II. Sub-samples of 5 Apples

Having examined the causes of variation between whole graded samples, it is now necessary to separate these into their component parts, to see how far size, colour and position of the fruit on the tree have influenced core-flush.

Table X summarizes the data for the two rooms. In this Table each entry is the mean per sub-sample of 5 apples. The second part of the analysis of variance is carried out in Table XI. In each room there were 768 sub-samples; there are therefore 767 degrees of freedom. Of these, 63 have already been allotted to differences between whole samples. Of the remaining 704, 11 are appropriate to comparisons of size, colour, position on tree and their interactions, and 165 to the interactions of kind of fruit with different trees, leaving 528 for error.

In this part of the analysis the first part is summarized at the head of the Table for comparison of the error variances, the estimates of variance in Table IX being now divided by 12 to reduce them to the same basis as the sub-samples. It is at once obvious that differences in colour and size have had an immense effect upon core-flush. In both rooms there was nearly three times the amount of core-flush in the green apples as in the red, while the largest fruits had over twice the amount of those in size grade 3. There is a

TABLE X

Effect of Size, Colour and Position on Tree upon Amount of Core-Flush

Mean amount in a sample of 5 apples

Colour	Red		Green		Mean	Red		Green		Mean
	Room 36° F.					Room 32° F.				
Position on Tree	Ter-minal	Spur	Ter-minal	Spur		Ter-minal	Spur	Ter-minal	Spur	
Size.										
Grade 1, 2¼–2½ in....	1·54	2·15	4·61	4·81	3·23	4·78	5·52	9·89	11·13	7·83
,, 2, 2–2¼ ,, ...	0·89	1·40	2·76	4·29	2·34	2·09	2·91	6·94	8·15	5·02
,, 3, 1¾–2 ,, ...	0·68	1·12	1·95	2·37	1·53	0·73	1·56	3·70	5·18	2·79
Mean (all sizes) ...	0·97	1·56	3·11	3·82	—	2·54	3·33	6·85	8·16	—
	1·26		3·46		2·36	2·93		7·50		5·22

TABLE XI

Analysis of Variance, Part II. Sub-samples of 5 Apples. Core-Flush

Variance due to :	Degrees of Free-dom	Room 36° F.			Room 32° F.		
		Vari-ance	" Z "	S.D.	Vari-ance	" Z "	S.D.
From Part I.							
Between whole samples.							
Trees and room blocks ...	18	36·77	1·3827	·—	19·10	1·3171	—
Within trees	45	5·36	0·4197	2·315	8·72	0·4531	2·953
Total	63	14·33	0·9119	--	20·26	0·8745	—
Within samples.							
Colour	1	929·06	2·9975	·—	4,004·97	3·5178	—
Position on tree	1	81·45	1·7804	—	211·89	2·0482	—
Size	2	185·20	2·1911	—	1,631·93	3·0689	—
Interaction of colour and							
position	1	0·73	·	·	12·92	0·6495	—
Colour and size	2	17·64	1·5122	·	79·33	1·5570	—
Size and position ...	2	6·64	0·5266	—	0·50	—	—
Colour, position and size	2	10·86	0·7729	·	0·27	—	--
Trees and kind	165	4·20	0·2979	·—	6·37	0·2963	—
Error	528	2·32	—	1·521	3·52	—	1·877
Total	767	5·65	—	2·376	15·44	—	3·929

smaller but significant difference due to position of the fruit on the tree, apples from spurs having in both rooms more core-flush than apples from terminals.   Of the interactions the most interesting is that of colour and size, the significance of which in both rooms is apparently due to the fact that in the middle-size grade the red apples have relatively less core-flush, while the green apples have relatively more than would be expected if both behaved alike.   Too much attention need not be paid to the interaction of trees and

TABLE XII

Effect of Position and Winter Injury in the Orchard and Position in Room upon Scald

Amount of Scald per Sample of 60 Apples in Each Room

Room Block	Block	Room 36° F.					Room 32° F.				
		I	II	III	IV	Means	I	II	III	IV	Means
Orchard Block	Injury										
S.E.	1	24·6	33·2	36·0	28·5	30·6 } 35·1	71·5	66·7	60·1	76·3	68·7 } 62·7
	2	35·5	47·0	43·0	32·7	39·5	61·5	53·1	60·5	51·5	56·7
S.W.	1	31·7	29·8	36·0	29·7	31·8 } 49·1	90·5	92·0	81·5	94·5	89·6 } 91·8
	2	54·2	71·7	67·9	72·0	66·1	102·5	92·5	106·5	98·4	100·0
N.E.	1	15·6	28·5	35·0	37·0	29·0 } 45·1	69·8	61·0	70·5	54·8	64·0 } 82·7
	2	59·1	49·5	72·0	64·2	61·2	103·9	102·0	102·0	97·4	101·3
N.W.	1	37·5	39·5	35·0	32·0	36·0 } 33·7	89·8	95·5	88·3	89·5	90·8 } 74·5
	2	29·6	24·2	40·3	31·5	31·4	61·0	47·0	66·0	58·5	58·1
Mean		36·0	40·4	45·6	40·9	Gen. mean 40·76	81·3	76·3	79·4	77·6	Gen. mean 78·66

kind.   The value of " Z " obtained in both rooms is only just above the 5 per cent. point in significance, and is probably mainly due to variations in individual trees due to the imperfections of the sampling method.

Comparing the two rooms, it may be noted that there is a considerably greater range in room 32° F.   While the small red apples have scarcely more core-flush than in room 36° F., in the larger grades there is more than twice as much.

Comparing the accuracy within the two rooms, it will be seen that reduced to the same basis (*i.e.*, the mean of a 5-apple sample), the comparison of the sub-samples is more accurate than that of the whole samples in both rooms.   The standard deviation for comparisons between samples within trees in room 36° F. was 2·315 or 98·1 per cent., while that between sub-samples was 1·521 or 64·5 per cent., a significant difference, as the Z test shows.   In room 32° the two standard deviations were 2·953 or 56·6 per cent. and 1·877 or 36·0 per cent., again a substantial reduction.   The

ratios of the two pairs of variances give an estimate of the relative accuracy, in both cases just over two to one. This probably means that there were local differences within the large blocks of trays in store, which reduced the accuracy of the comparison between trays, and that therefore the sub-samples within the trays, which were closer together, were more accurately compared. The difference is, however, not so great as is sometimes found in agricultural experiments.[3]

Analysis of Scald Records

The analysis of the core-flush records having been given in some detail, it is not proposed to do more than outline the main causes of variation in amount of superficial scald. The process is identical, and the only difference is in the results.

Tables XII and XIII summarize these in the same way as has been done for core-flush. As before, there is a large difference in the two rooms, that with the lower temperature having nearly double the amount of scald.

TABLE XIII

Effect of Size, Colour and Position on Tree upon Amount of Scald

Mean Amount in a Sample of 5 Apples

	Room 36° F.					Room 32° F.				
Colour	Red		Green			Red		Green		
					Mean					Mean
Position on Tree	Ter-minal	Spur	Ter-minal	Spur		Ter-minal	Spur	Ter-minal	Spur	
Size.										
Grade 1, 2¼–2½ in .	1 17	1·17	6 07	5 59	3 56	3·77	3·80	8 91	9 61	6·52
„ 2, 2–2¼ „ .	1·02	1·00	5 76	5 60	3 35	3 87	4 07	9 12	9 28	6 59
„ 3, 1¾–2 „ .	1·27	1·21	5 75	4·90	3 28	3 41	3 68	9 80	9 31	6·56
Mean .. .	1·25	1 13	5 84	5 36		3 69	3 85	9 28	9 41	—
	1 19		5 60		3·40	3 77		9 34		6 55

With respect to differences between trees (Table XII), orchard blocks show significant differences in both rooms, the two blocks, N.E. and S.W., which had most core-flush, having also more scalp. Winter injury appeared to have a marked effect in these two blocks, the more severely injured trees having considerably more scald here than in the other two. To try to explain these results would be hazardous without more information as to soil and in default of a more accurate measure of amount of injury. The question whether a moderate amount of winter injury really affects the keeping qualities of fruit taken from apparently healthy branches must be left open. It is possible that the type of injury may be of

more importance than the total amount, and that, in consequence, the method of grouping the trees was at fault. It was unfortunate that so few completely undamaged trees were available for study. On the whole, it seems likely that the fruit from slightly injured trees keeps as well as that from quite healthy trees.

Once again there are considerable differences between apparently like trees, which are not accounted for by the above factors. Since these trees form part of an ordinary commercial orchard, and are of unknown history, this is hardly surprising.

The effect of position within rooms is not marked in either storage-chamber, though it is barely significant in room 36° F., in which block I had rather less scald. It is an interesting fact that blocks III and IV, which were examined for scald at the same time as for core-flush, and which developed definitely more of the latter disorder, were not noticeably higher in amount of scald.

The accuracy of the comparison of whole samples was much the same as for similar amounts of core-flush. In room 36° F. the mean amount of scald was 40·76 and the standard deviation, after eliminating differences between trees, 7·88 or 19·3 per cent. In room 32° F. the general mean was 78·66 with a standard deviation of 9·27 or 11·8 per cent. That these samples were again not homogeneous is shown by the effects of colour, size and position on tree in Table XIII. These results, however, unlike those for core-flush, show no effect of size or position on the tree. Colour is apparently the only factor, of those included, which has had an influence upon superficial scald, the green apples having very much more than the red.

Comparing the accuracy of comparisons between whole samples and between sub-samples, the sub-samples in this case only give slightly lower values of the coefficient of variability, 16·2 per cent. as against 19·3 per cent. in room 36° F., and 10·2 per cent. as against 11·8 per cent. in room 32° F., and in neither case are the differences significant. It appears, then, that variations in the storage-chamber have made little difference to the amount of scald.

It is evident from these results that colour and size of fruit were predominant causes of variability in the amount of core-flush, and colour in the amount of scald, to develop in these McIntosh Red apples, and probably account very largely for the high variation in the random samples. Some of the variation of the graded samples between trees is evidently due to these causes, for, as has been pointed out, the grades are fairly wide, and where trees differed markedly in colour or size, the process of grading did not eliminate the difference altogether. The effect of size of fruit on core-flush is not surprising, since it has already been shown by several workers

that size is of importance; but the effect has not always been the same, for though larger fruit have been shown to have more internal breakdown,[16, 21] Wallace, working at Long Ashton, found more core-flush in the smaller fruit of Bramley's Seedling.[21] The definite difference between the red and green apples was less expected, as it was thought previously that this was of relatively small importance compared with ground-colour, though green apples were known to suffer more from scald. It is evident that more attention will have to be paid to this character in the future. The amount of scald which developed was probably due to the fact that as a whole the fruit was rather under mature when picked, since McIntosh when mature is said to be not very susceptible to this trouble.[4] It seems likely that there is some connection between maturity and amount of red colouring, and this may be the reason for the comparative resistance of the red apples.

Even though these characteristics of the apple are of evident importance, it seems likely that there are other factors in the orchard which have their influence upon tree to tree variability. Exactly what these factors are it is difficult to tell, but the experiment makes it clear that if accurate storage trials are to become a practical proposition, every effort must be made to obtain trees which are as uniform as possible.

Distribution of the Coefficient of Variability

In considering further the variability of this material, the fact has been observed that in room 32° F., where there was more core-flush and more scald, the coefficient of variability was also much lower. This suggested that, as might have been expected, the coefficient of variability decreased as the mean increased. With the rather large differences between the various kinds of apple in different sub-samples, it seemed probable that all sets of sub-samples would not have the same coefficient of variability. In order to test this, the core-flush data were re-analysed for both rooms, taking each colour and size-class separately. There were thus obtained six values of the coefficient of variability for each room, from sets of samples which gave mean amounts of core-flush varying from 21·0 per sample of 10 apples for the large green apples in room 32° F. to 1·8 for the smallest red apples in room 36° F.

The resultant coefficients of variability, after eliminating the effect of differences between trees and position in the room, adjusted to a whole sample (60 apples) basis are plotted in Fig. 2. It will be seen that these points approximate rather nearly to a curve drawn on the assumption that the samples conform to a binomial

distribution. If such were the case, the standard deviation would be $s = \sqrt{\bar{x}q}$, where $\bar{x}$ is the mean amount of core-flush and $q = \dfrac{180 - \bar{x}}{180}$. The coefficient of variability, which is the standard deviation as a percentage of the mean, would then be $\dfrac{100 \cdot s}{\bar{x}} = 100\sqrt{\dfrac{q}{\bar{x}}}$.

Actually the points tend to lie rather above the curve. This is accounted for by the fact that the sub-samples are not homogeneous, and the greater the amount of variation within samples, the larger the departure from the curve which may be expected. Thus the
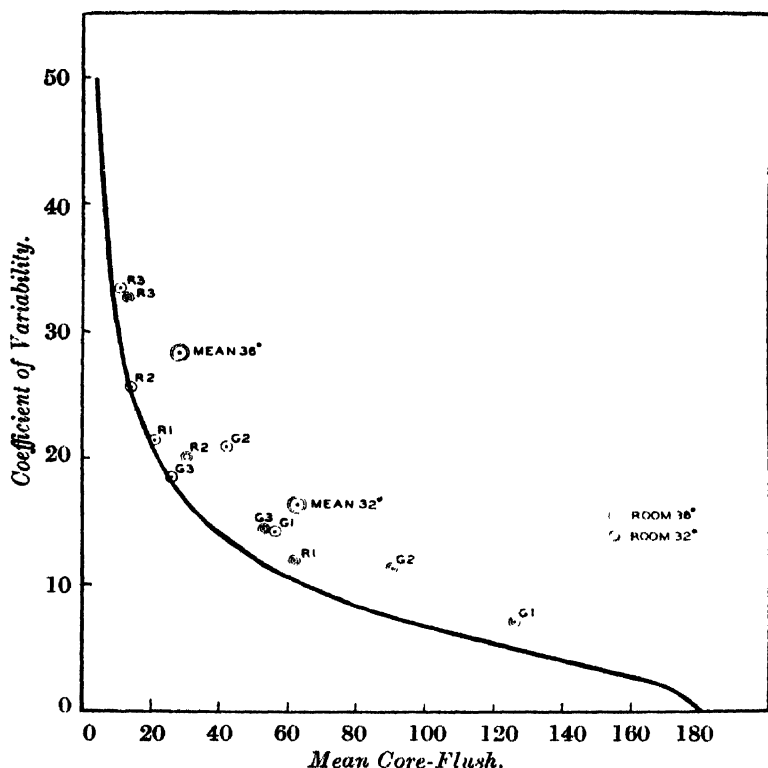
FIG. 2.—Distribution of Coefficients of Variability for Core-Flush in Samples of 60 apples.

R1 = Red apples, size grade 1.   G1 = Green apples, size grade 1.
R2 =   „   „   „   „   2.   G2 =   „   „   „   „   2.
R3 =   „   „   „   „   3.   G3 =   „   „   „   „   3.
Mean 36° = all samples in room 36° F.
„   32° =   „   „   „   32° F.

coefficients obtained for the whole of the two rooms are rather higher than where the biggest differences are separated out Higher still are values obtained for the random samples where no attempt was made to obtain uniformity within the sample.

A similar result was obtained by van der Plank in an experiment on wastage in oranges,[20] though in this case only the number of affected fruits was considered, no account being taken of differences in degree.

This illustrates the importance, in the first place, of eliminating large differences from an experiment in which it is designed to show comparatively small ones.

Secondly, where the results of the experiment depend largely upon a single examination of all the fruit, it is evidently necessary to include a series of extra samples, representative of all the fruit in the trial, for periodic examination with the object of attaining an average amount of breakdown for the whole trial, which will give a reasonable uniformity without going so far as to mask differences between treatments.

With samples of 60 apples and material such as that in the present trial, graded to eliminate large differences within samples, it seems that an average amount of core-flush of from 60 to 80, or a little more than one third of the total possible, would have given the best results.

Such a degree of uniformity would, of course, only be attained were it possible to design an experiment so that each treatment was carried out on a uniform sample taken from each tree. Where the experiment consists in comparing the fruit of different trees, the problem is more difficult, and will be further discussed later.

DISCUSSION

Having examined some of the causes of variability in certain storage disorders of McIntosh Red apples, it remains to use the information to formulate methods of sampling for use in future experiments.

Whether or not the particular results obtained with regard to effects are of general application does not affect the issue. In other varieties, climates and conditions, the effects may differ; but the fact must be borne in mind that in storage experiments differences between trees in the orchard and between such characters as size and colour of the fruit on a single tree may be the cause of large differences in keeping quality.

The first point of importance is the necessity for a greater uniformity in the trees used for such experiments. As long as very large differences remained undiscovered, the actual source of the material and its variability were not vital. Provided sufficiently large samples were obtained, results could be demonstrated with a very simple sampling technique. But experimental work with apples has now reached a stage when finer shades of difference are

being sought, and for this purpose greater uniformity is essential. In horticultural research of the past decade a great deal of work has been done to attain this end, though generally with characters other than storage qualities as the object. With the increased interest in this aspect of commercial fruit-growing, the provision of suitable fruit must be borne in mind in the layout of new experiments, the produce of which may be subjected to storage tests. At present very little such material exists. Apple orchards take many years to mature; and the number of experimental plantations laid down in such a way that some of the sources of error here examined can be controlled, and which are as yet old enough to provide material in quantity, is still very small.

In the meantime storage work must proceed and available material must be utilized.

It is not possible to lay down a technique of sampling which will suit all experiments; as in other branches of work, the sampling method must be designed to suit the circumstances of the experiment. It is only possible, therefore, to outline one or two hypothetical experiments, and to suggest a method for use in each.

Suppose that the experiment concerns only differential treatment of a single variety after the fruit is picked. Such treatments may be different conditions in the store itself, or before or after the fruit is stored. The material may be of known or unknown origin. If known, the first effort should be to use only trees which are as alike as possible. Situation, age, crop, rootstock, disease, etc., should all be taken into account, and their effects either eliminated or included in the experiment. If the latter can be done, the experiment will have a wider application. As in a field experiment with a factorial design, each class of tree should be subjected to each storage treatment.

The material may, on the other hand, be of unknown origin. In either case, as careful a grading for size and colour as is consistent with a minimum delay in storing should be carried out, and where possible individual trees should be kept separate.

Once this grading is done, the more grades which can be included in the experimental design the better. If only one grade is includde, some four to six samples of 50 or 60 apples, each subjected to each storage treatment, should be sufficient to prove differences of about 20 per cent. in, say, core-flush, if the average level of all treatments is not too low. If, as is possible under some Canadian conditions, the crops are large enough to give to each treatment a sample from each tree, so much the better; but if, as is more probable, they are not, the trees should be divided into groups, the members of each group being as near to or as like each

other in some other way as possible, and the crops amalgamated. In this event each treatment should receive a sample from each group of trees, the sample being composed of an equal number of fruits from each tree in the group; the more uniform the fruit in the sample, the less the replication of samples necessary, but the number should not be less than four.

The experiment will be improved if more than one grade of fruit is included. In this case the replication of samples of the same grade can be reduced, but it would not be advisable to have less than duplicate samples for each grade of fruit under each treatment. In the actual arrangement of samples, the possibility of time, positional or other outside factors causing variation should be remembered, and the experiment arranged accordingly. Thus, if several grades of fruit are included in a number of storage-rooms, the trays or boxes should be arranged in blocks, each block to contain one sample of each grade. When examined, the period of examination should be spread over all samples, so that one or another is not unduly favoured.

If the criterion by which the experiment is to be judged is of the same nature, the progress of the fruit should be carefully watched, by some such method as was used in the experiment herein described; and if only one examination is made, a definite point to be reached must be decided upon before any fruit is removed. This point will depend upon the homogeneity of the samples. If they can be assumed to be as uniform as those described in this paper, the coefficient of variability is likely to be rather higher than that which would be obtained with a binomial distribution. In the present instance an average figure of from 30 to 40 per cent. for core-flush or scald would be likely to give good comparative results.

The problem of elucidating the effect of orchard practices upon storage qualities or life is more complex and demands a different technique. Such practices may have a direct or an indirect effect upon the behaviour of the fruit in store. Thus it is possible that externally identical apples with a different history may behave differently in store. On the other hand, the orchard practices in question may produce fruits which differ in size, colour or in some other quality and which thus have different potentialities in store. It is thus essential that samples for storing should represent the apples of the trees from which they come; uniform samples can at best present only a part of the picture.

This type of experiment is evidently very much more difficult, since, as has been shown, the fruit from a single tree is very variable. To meet this difficulty the method of selecting a " typical " sample has been evolved. This, however, as has been stated previously,

demands very considerable skill on the part of the research worker. To the writer it seems that a method of random sampling will be safer and capable of wider use. That these samples will be variable is evident, but provided that the size and colour of the apples in the sample are accurately recorded and the degree of affection is not too low, there does not seem to be any reason why quite accurate results should not be obtained.

Unfortunately this was not foreseen in the design of the present experiment, and the size and colour of the apples in the random samples were not recorded, except for a few notes on exceptional trees. It is therefore not possible to show what degree of accuracy might have been attained if their effect were eliminated in the analysis of the results. It seems likely that there would have been significant correlations between size or colour and amount of core-flush, for example, and the analysis of co-variance could have been used to eliminate their effect. By this means it would be possible to estimate the direct effect of any orchard practice upon keeping quality, without losing the information concerning the indirect effect through such characters as size and colour of the fruit.

The actual size of the sample might need adjustment in such an event, for it must also be large enough to sample adequately the size and colour of the fruit on the tree. Information as to the proportion of fruit needed has been given for English conditions,[7, 10,] but this will not necessarily be the same for the larger trees usual in Canada.

In conclusion, it should be emphasized that while the main principles of sampling herein suggested should form the basis of any particular method, the actual scheme to be adopted must depend upon the conditions of the trial. In the two examples discussed here tentative methods are suggested, but only by trial under the conditions of an actual experiment can their value be determined. In horticulture there are no hard-and-fast rules, and only experience can dictate the best course to pursue in any given set of circumstances.

SUMMARY

1. An experiment carried out on McIntosh Red apples in Ontario in the season 1936–37, designed to throw light upon causes of variability in the keeping quality of stored fruit, is described.

2. Six samples of 60 apples each were obtained from each of 32 trees in a commercial orchard. The trees were selected to show the effect of differences in position in the orchard and of degree of winter injury.

3. Two of the 6 samples were selected from the trees at random; the other 4 consisted of 12 sub-samples of 5 apples each, taken to represent different categories for size, colour and position of the fruit from each tree.

4. Apples from 16 trees were stored at a temperature of 36°F. in one room and apples from the other 16 in a similar room at a temperature of 32° F. The samples were so arranged that 1 sample from each of the 16 trees was placed in each of 4 blocks occupying different positions in the rooms.

5. The fruit was removed in June 1937 and records obtained of core-flush, scald, fungal rotting, shrivelling, etc.

6. The data for core-flush and scald are analysed and the effects of the various factors in the experiment are shown.

7. In room 32° F. there was a considerably greater amount of both core-flush and scald than in room 36° F.

8. Differences between trees in the orchard were very marked both in the random samples and in the graded samples, but the effects of position and of winter injury were not very clear, possibly owing to the limitations of the experimental design and available trees. There was a notable tendency for samples from the same tree to be more like one another than those from different trees, especially in the graded samples.

9. Size, amount of red colouring, and to a lesser extent position on the tree all had an influence on core-flush, but only red colouring was of importance with regard to scald.

10. The variation between samples is shown to depend to a large extent upon the average amount of core-flush or scald present in the samples, those with small amounts being much more variable than those with large. The distribution of the coefficients of variability obtained is compared with those expected for a binomial

distribution. While evidently of a similar nature, those obtained were rather higher, the nearer to complete homogeneity within a sample, the closer the approximation to the binomial curve.

11. It is concluded that in designing an experiment to show fine differences, care must be taken to eliminate first the larger differences. Further, it is essential before the main examination is begun to fix carefully the level to which, on the whole, breakdown is to be allowed to proceed.

12. Methods of sampling for use in certain contingencies are discussed in the light of the experience gained in this experiment.

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DISCUSSION ON MR. HOBLYN'S PAPER

Dr. BARTLETT : It is some years since, with one or two members of Jealott's Hill Research Station, I went down for the day to East Malling in order to learn something from Mr. Hoblyn on the relation between statistics and horticulture : we went over the experimental apple orchards there, went indoors and looked at the sorting of apples, and then discussed the actual results of an apple experiment. I have ever since associated apples with Mr. Hoblyn, and am therefore interested to observe that even when he gets to Canada that association still holds.

Mr. Hoblyn has never been slow to apply the principles of factorial design to horticultural experiments, and we have here an interesting example not of an application to field experiments, but to the study of the keeping quality of apples in store. The problem is in this instance a uniformity trial : it is not a field experiment in the sense of actual treatments used in the orchard, but the factorial design is still there very strongly. The fact that it was a uniformity trial, and that you had to take the material as you found it, might have tended to impose certain limitations, but Mr. Hoblyn tried to get over this as effectively as possible. If apples of different colour and size are taken indiscriminately, it may be found that after the apples have been sorted for size, they have also inadvertently been sorted for colour. But after taking the random samples, Mr. Hoblyn took the rest of the apples from each tree and selected simultaneously, as far as the material allowed, for colour, size and so on. This tended to make the analysis given later in the paper legitimate.

The specific criticisms I have to make are trivial. In connection with Table IX on page 147, the significant difference showing the effect of position in the room is interesting; my concern, however, is with the part of the table that splits up the tree classification, as the notes in the context on orchard blocks and injury appear slightly curious. Surely since the " between-tree variance " is certainly as large as the other " tree variances," there is no real significance in the interaction term, as this should only be compared from the point of view of significance with this variance (the second from the bottom of the table).

The subjective element in grading (which comes in here, for example, in the grading for colour and in the estimation of scald)

G 2

raises an interesting general problem. In this experiment standards were put up, and I have no doubt that the grading was accurate. I have been more doubtful sometimes when dealing with work from abroad, such as the grading of oranges, or the tasting quality of coffee.

In an experiment where articles have been treated in some way, and quality is considered, it is worth noting that if those articles are graded in ignorance of the (random) order of treatments, then, however badly the grading may be done, it will not affect the validity of the experiment. It will only mean that the more accurate the grading, the more accurate the experiment.

Another interesting point is the suggested use of covariance (p. 158). As I see it, you would have, in your random sample from the manuring experiment, to test out the direct effects of manuring treatments, then study the regressions from colour, size and so on, and then see how far direct manuring effects were eliminated; they might be wholly eliminated, but if only partly, it would be necessary to be more careful when trying to separate (1) the effect of manuring in so far as it affected colour and size and (2) other manuring effects.

I congratulate Mr. Hoblyn heartily on his paper, and have much pleasure in proposing this vote of thanks.

MR. TIPPETT : 1 have great pleasure in seconding this vote of thanks to Mr. Hoblyn. I endorse all Dr. Bartlett said about Mr. Hoblyn's illuminating demonstration of the use of complicated arrangements and statistical analyses to the study of variations that may occur in samples, and to the devising of sampling techniques. One topic he touches is of fairly wide interest—namely, how far should the deterioration of the apples go before the investigator stops, cuts them up, and measures the results of the experiments? Mr. Hoblyn has ventured suggestions on p. 157. He says, "In the present instance an average figure of from 30 to 40 per cent. for core-flush or scald would be likely to give good comparative results." That sort of problem arises in various fields. For example, in order to test consignments of glass bottles for their ability to stand the rough treatment they may get in transit, an experiment is designed to subject the bottles to an action simulating that treatment, and it is necessary to decide what proportion of bottles should break before the treatment is stopped and the broken bottles are counted. Possibly there may be more in that problem than Mr. Hoblyn suggested. I do not see how a uniformity trial alone can solve it.

First, if it is desired to compare the keeping quality of two varieties of apples, A and B, A may be better than B in the early stages of deterioration, but, later, B may be better than A. If one is commercially interested in the keeping quality up to an early stage of deterioration, a useful comparison would not be obtained by allowing the deterioration to go a long way. That is a technical and not a statistical factor that may determine within what limits deterioration should be studied, and information on this can only be obtained by comparing typical varieties at different degrees of deterioration.

Apart from that, if that factor is not important, then how far should one go ? There are two elements that make for accuracy of comparison : (1) the random error and (2) the difference between the two varieties at the different stages of deterioration, and it is the ratio of that difference to its standard error that has to be made a maximum. In comparing varieties *A* and *B*, it is possible that the ratio of the difference to its standard error may be greater at 80 per cent. of deterioration, say, than at 40 per cent., although the standard error may be least at the 40 per cent. deterioration. Again, the best degree of deterioration can only be determined by comparing typical varieties at different degrees.

It does seem to me that in determining this kind of question it is necessary to study the interaction between the degrees of deterioration and the kind of effect one is wishing to measure, whether the effect be a comparison of varieties or of manurial treatments, before it is possible to say what is the best degree of deterioration at which to stop the experiment. It may be that I am under a misconception, and that, not having read the paper carefully enough, I have missed some point made by Mr. Hoblyn ; but I should be very grateful if he would say something about the question I have raised in his comments, and say exactly how he arrived at the 30–40 per cent. criterion. It sounds as though I have been critical, but my criticism is not general. I have very much enjoyed hearing the paper, and much admire the way in which the work has been done. I therefore second the vote of thanks with sincerity.

Mr. van Rest said that Mr. Hoblyn's paper seemed to him to be an excellent attempt to rid one kind of horticultural experiment of the bugbear of variation. Mr. Tippett had, in speaking of the choice of time at which the measurements should be made, opened up a subject on which he (the speaker) proposed to enlarge. This subject did come within the province of a statistician, as he was often presented with a set of data about which he could only say that the variability was too large to show any of the effects which the experiment sought to test. If he had the opportunity, as he had when he himself was the experimenter, he could often considerably reduce the variation in an experiment by suitably choosing the quantity to be measured. He had an example of this from his own experience in tests involving the fungal decay of wood. The measurement made was usually loss of weight after some fixed period, and the purpose of the tests was to show the relative toxic values of different kinds of wood or of agents added to the wood. The results often exhibited extreme variation even between pieces under supposedly identical conditions. Discussion revealed the observed fact that these differences were in part due to varying latent periods before the attack " got hold." Thus it appeared that a measurement either of this latent period or of the rate of loss of weight at a late stage (when the rate was nearly linear with time) would have less variability than a combined measure. These measures could easily be translated back to practical requirements, and would by their separation afford more precise experiments into differences of tox-

icity. No figures were yet available, but such an example did at least suggest that the more obvious measurement was not necessarily the most suitable for a given test.

MR. COCHRAN, referring to Mr. Bartlett's remarks·about the subjective element in grading, did not think that that element raised any difficulties or doubts about the interpretation of Mr. Hoblyn's experiment; there were, however, cases in which it might do so. For instance, in an experiment on the effects of storing for different periods of time, material graded by eye on one date would be compared with material graded at some later date. There was a real danger that the standard of judgment might have changed unconsciously between these dates, and in such cases it was advisable wherever possible to have some objective check on the grading.

Further, in an experiment which was completely graded at the same time, it was essential that the grader should be unaware in what order the " plot " yields were presented to him, particularly when the experimenter himself carried out the grading. If this condition were satisfied, the verdict of the analysis of variance could be relied upon, though if the grading were poorly done, real differences between treatments might be missed. Mr. Cochran then gave an example of the large differences in ability which might exist between graders. He had been one of a party of three who graded a pot experiment on clover, the pots being assigned grades between o and 5 (by half-units) by comparison with five standard pots. The different graders agreed well about the treatment effects, but the poorest grader would have required twice the number of pots to obtain the same accuracy as the best grader. Incidentally, the best grader had no connection with the experiment, while the poorest was the experimenter himself.

DR. WALLACE congratulated Mr. Hoblyn for carrying out this trial. He himself had been concerned with the effects of different treatments on storage quality more than with the type of experiment on which Mr. Hoblyn had been engaged, and he knew how difficult the matter was purely from the practical side. He could not add anything on the statistical side to the paper, but there were several horticultural and storage points he would like to bring forward.

In the first place, on p. 138 it was stated that the experiment took five picking days. This was rather a long period when apples were ripening, as there were bound to be differences due to maturity, and in developing an experimental technique something must be done to shorten the time.

On p. 140 Mr. Hoblyn attributed the good keeping of the apples in the experiment to the careful way in which they were handled; he himself would attribute it to the fact that the fruit was picked immaturely. Again, Mr. Hoblyn stated that he had effected more breakdown by lowering the temperature, whereas Dr. Wallace thought it was due to the extra month given to the fruit before examining them again after the temperature changes were made.

If temperature were lowered (*a*) it would increase the numbers of fruits liable to low-temperature breakdown but (*b*) it would decrease the rate of chemical change, which would also decrease the rate at which breakdown would develop. Probably with the 36° F. change many fruits passed into the zone of low temperature, although it was doubtful whether at the stage at which the fruit was moved that point would be affected.

In Table II on p. 141 the random samples and the graded samples seemed to have reversed the results at the low temperature from those at the high temperature. The key to this anomaly might be found in the fact that in the random samples a greater proportion of fruit might be susceptible to low-temperature breakdown. A good parallel was when potash-treated fruits were compared with potash-deficient fruits. It was often found that at higher temperatures the low potash fruits kept much the best, whereas when subjected to a temperature where low-temperature breakdown occurred the result was completely reversed.

He rather doubted the statement on p. 147 that one day was responsible for the enormous increase in core-flush. Core-flush did not develop at such a rate.

With regard to the data in Table X, the suggestion was that the differences were colour differences between red and green fruits. If, however, exposed fruits from the outside of a tree and shaded fruits from the inside were compared. they would give the same type of result as that recorded here. The exposed fruits would give less rot and less core-flush, and were more susceptible to bitter fruit, thus providing another aspect which ought to be considered in such an experiment.

On p. 152 it would be found that the core-flush and scald did not run parallel, and he could endorse that. There was often a curious effect with variables of fruit which were highly susceptible to core-flush namely, that if scald were induced by a factor such as bark-ringing, core-flush would often not develop, but flesh breakdown just under the skin would follow the scald.

On p. 153 some results were given of Dr. Wallace's work at Long Ashton, where he found more core-flush in the smaller fruit of Bramley's Seedling. Varieties, however, differed in this respect, and Mr. Hoblyn seemed, in measuring core-flush, to have included some breakdown in the flesh area, still calling it core-flush. In Mr. Hoblyn's experiment it was found that green apples developed more scald than red ones, which was a usual finding, but there were exceptions. For example, if trees of Newtown Wonder (the fruits of which are highly susceptible to scald) were bark-ringed, both scald and colour would be greatly increased, and this raised interesting points with regard to colour and maturity. There appeared to be some confusion with regard to the whole question of colour and maturity; was a red-ringed fruit more mature than a green one? Evidence from the usual tests was conflicting. The starch test showed more intense coloration with ringed fruits than with not-ringed greener ones, because initial starch content was higher; the dry matter, sugars and starch, might be 1 or 2 per cent. higher

than in the not-ringed fruit. If, in the same way, one examined the difference between an exposed (red) and a shaded (green) fruit from a single tree, it would be found that they were totally different types of apple. The green fruit would be higher in acid and lower in dry matter : differences could be detected by testing, and there was a difference in texture.

All these were purely horticultural points, but a great deal could be done by a knowledge of these matters in conducting an experiment such as this.

MR. HARTLEY said that the paper had been particularly interesting to him, as many statistical problems dealt with had an analogy in another branch of agricultural experimentation with which he had been concerned until recently—experimental work with poultry. This could best be made clear by a brief description of a particular example of such an experiment—a fattening experiment with table poultry which was representative of a certain type of design frequently employed.

Four groups of table birds were reared on four different rearing rations, denoted by the letters A, B, C, and D. At the end of rearing the fattening experiment proper began. Not all the birds were suitable for fattening, and it frequently happened that even from the suitable birds of the four rearing groups a representative sample had to be selected for fattening. These were then accommodated in a dark shed—the fattening-shed—for a short period.

The analogy to Mr. Hoblyn's storage-experiment was clear. The birds corresponded to the samples of apples, the four treatments A, B, C, and D, on the rearing ground, to the various treatments in the orchard. In both cases samples had to be selected from the experimental material and had to be accommodated in rooms, where, as would be seen, similar problems of local control had to be solved. The analogy went even further. Just as the samples of apples were graded, so the samples of birds, selected according to their fitness for fattening, were further graded, and normally the body-weight of the birds was taken as a criterion for grading. Thus there were three weight classes, heavy (H), medium (M), and light (L).

It would at first appear unlikely that the position of the birds in the fattening-shed would have an effect on the fattening results. Experience, however, had shown that it did. It was unfortunate that Mr. Dudley, the statistician to the National Poultry Institute, where these experiments were carried out, was not able to be present at the meeting, as he would have been able to testify from long experience that it was certainly worth while to make allowance for a possible effect of the position in the fattening-shed. Again, there was a point of similarity between fattening-shed and storage-room, and correspondingly they had now to consider a design which introduced local control in the fattening shed. The unit there was the coop : a cage consisting of three compartments holding about four birds each. This suggested a straightforward " split-plot " allocation of treatments. The coops played the part of blocks, whilst the three compartments corresponded to the trays of Mr. Hoblyn's arrange-

ment. In each coop weight grades *H, M, L* were allotted at random to the compartments as main treatments, and then in turn in each compartment rearing treatments *A, B, C,* and *D* were randomized.

Mr. Hartley said he had certainly not given a complete description of the experiment, but he hoped it was sufficient to show the similarity between the two problems. The split-plot technique appeared to be the appropriate design in either case.

He was not quite clear, however, on a certain technical point in Mr. Hoblyn's design. In Table VIII a tabular arrangement was given of the design for the graded samples, row-headings denoting the eight treatments of the " between pairs of trees " analysis—viz., all combinations of orchard positions and injury category. The four replications of treatment—say, S.E., 1—were shown in the first row. These figures represented the amounts of core-flush of four pairs of samples from the *same pair of trees*, but, nevertheless, they served as *four replications* of the treatment S.E., Injury 1.

Statisticians who, like himself, did not know much about experimentation with fruit trees, might be inclined to compare this pair of trees, from which four samples had been drawn, with a plot in a field trial, allotted to a certain treatment, from which four quarters had been harvested to obtain replications. It was known that such quarter-plots were not proper replications : therefore, it would be most helpful to have Mr. Hoblyn's expert opinion on this point, in order to throw some light on the corresponding problem in fruit experimental work. Mr. Hoblyn seemed to be interested in two different questions, viz. :

(*a*) The question of whether fruit from different *individual* trees differed in their keeping quality.

(*b*) The effect on keeping quality of tree *characteristics* (such as winter injury).

In a test for the first problem, samples from the same tree were the proper replications to use, but for the second problem one would prefer discrete trees as replications for the analysis.

In conclusion, Mr. Hartley offered tentatively a suggestion for a slight modification of the design, in which discrete trees occurred as replications :

There were eight trees in each Orchard block, four having injury one, and four injury two, and each tree had four graded samples, With Mr. Hoblyn's design *all samples* from four of these trees were placed in one room and *all samples* from the remaining trees in the other room. But could not two samples from *all trees* be placed in one room and the remaining two samples from *all trees* be placed in the other room ? Then each of the eight treatments would be represented by four-tree replications in each room. Trees and samples would change their rôle.

The suggestion was not put forward in a spirit of criticism, but in the hope that it might provide a means of possible improvement. The expert must decide whether the modifications were practical. Finally, he wished to express his heartiest thanks to Mr. Hoblyn for his most interesting paper.

MR. CANNON said that in storage work at Ottawa which had been going on for the past five years, one of the chief difficulties met with had been the large variability occurring within samples. Until Mr. Hoblyn's visit to Canada they had been able to prove only larger differences, and were getting to a point where it was becoming necessary to show differences of a smaller nature. A number of factors which Mr. Hoblyn had shown to have contributed to this variability had not been accounted for in previous experiments, for example, the effect of colour on core-flush. They had, however, as far as scald was concerned, recognized the fact that more scald usually developed on green apples than on red. Another important point brought out was the effect of size on core-flush development. In storage trials with McIntosh Red, they generally used core-flush as an index of keeping quality, particularly when comparing fruit grown under different cultural and manurial treatments. Another interesting thing they found was that different positions in the room caused differences in keeping. It had previously been considered that there would be little or no differences within a single room because in the centre of each room a rotating fan was kept going continuously while the fruit was in store. Another point Mr. Hoblyn's paper had brought out was the necessity for determining the proper time to make an examination of the fruit. Previously the samples had always been examined at three different times throughout the storage season, and sometimes even at the second examination it was found that there was not a sign of core-flush or scald. Again, there was the possibility of allowing the fruit to remain in store too long, so that treatment differences would be masked to some extent.

Mr. Cannon thought Mr. Hoblyn had certainly brought out many very interesting and important considerations, and they at Ottawa were all greatly indebted to him for this paper.

MR. DULY said that this was the first time he had ever listened to or read any account of a statistical investigation of any kind, and he was afraid his observations on the statistical side were only to say how amazed he was to see how the old saying Ein Versuch ist kein Versuch had been developed. He found the results interesting, and in some manner surprising. He himself had very little opportunity of carrying out investigations on fruit, but he had frequently examined the condition of large consignments of apples arriving in this country from overseas, and the one observation he would like to make regarding this particular paper was that in this examination he did not remember at any time having found examples of scald on apples at the same time as internal breakdown, at least to any extent. It seemed to him that these two characteristics were never associated in the same apples.

Mr. Duly said he had much enjoyed an interesting evening : the paper and discussion had shown to him an entirely new vista of possibilities.

MR. HOBLYN, in reply, said : A large number of points have been

raised, and I must apologize if I have not quite grasped all of them. I will try to deal with them.

I quite agree with what Mr. Bartlett said about Table IX, and, as I said later on, I think that particular interaction was due to chance, and was indistinguishable from the " Between Trees " Variance.

Grading is a very interesting point, and one which crops up in every branch of horticultural work. To be able to grade accurately by eye is by no means easy, and when different workers are employed, very often entirely different results are obtained. With five people grading for colour you may get completely different results from each. We tightened this grading up until I think the colour and other grading were fairly fool-proof : I did the whole of the colour grading myself, and I do not think that the grades varied appreciably.

As regards the use of the analysis of co-variance, it was a great pity that I had not the data to examine its possibilities, as unfortunately we did not grade the random samples for colour and size. I hope I may one day have another opportunity.

As to the question of how far the fruit should be allowed to go before starting grading, there is the same difficulty in other branches of horticulture, and statisticians have not perhaps paid enough attention to it in the past. I hope this will impel people who have the same kind of problem to go into the matter in more detail than I have been able to do here, and give us a lead. My suggestion of 30 or 40 per cent. was based on the curve given in Fig. 2. Beyond this point there was likely to be little further reduction in variability, and sufficient core-flush would have developed to give reasonable comparative results.

With regard to the interesting question of deciding what is the best variable to measure, we measured the total amount of core-flush, and an instance was given of taking the total loss of weight in wood samples, where perhaps the rate of loss was a very much less variable character. I agree that time spent in finding the best criterion to measure is never wasted, and it is a point always worth considering in experimental design. People are too often apt to design their experiments first and think what they are going to measure afterwards.

A great many points are raised by Dr. Wallace in the interpretation of the experimental results. I will not quarrel with him, as he knows much more about storage experiments than I do. To the present thesis the important point is that all these things may matter; whatever the cause and effect, they are possible causes of variation which should be remembered and allowed for in the experimental design.

I am grateful to Dr. Wallace for his remark concerning my definition of core-flush, and for drawing my attention to a misconception on my part through not being present when the grading took place. I have accordingly adjusted the description in the text.

I am much interested in the poultry experiments on much the same lines, which were explained to us. We did consider placing fruit from each tree in both rooms, and the reason we gave it up was that

it was practically impossible to do it. Further, with only two samples, very little information with regard to position within the rooms would have been obtained. This experiment took a lot of organization to get the apples into the two rooms in their right place within twenty-four hours of picking. A good many things had to go, and I think that was one of them.

NUTRITIONAL STUDIES OF CELERY IN RELATION TO CERTAIN PHYSIOLOGICAL CHANGES IN COLD STORAGE[1]

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INTRODUCTION

Considerable experimental work has been done on the production of celery in the field.   Experiments are being carried on year after year in an attempt to find which fertilizer or fertilizers produce the best results.

The celery acreage for the Province of Quebec has increased from 169 acres in 1930 to 760 acres in 1936.   McKibbin and Stobbe (14) have shown that there are 40,000 to 50,000 acres of peat and muck soils within a radius of 45 to 65 miles of Montreal.   Most of this area could produce good celery.   However, figures show that for the years 1930–35 an average of $278\frac{1}{2}$ cars of celery were imported each year into Montreal.

It has been shown that celery of good quality can be grown in Quebec. The weak point at present is to keep the produce in good condition in the storage until it will bring a high price or until it is required for consumption. With this in view the Quebec Refrigeration Committee undertook a rather extensive celery fertilizer experiment last summer near Ste. Clothilde de Chateauguay to determine if the different levels of nutrients had any effect on the keeping quality in cold storage.   After the crop was harvested and the records of yield taken, from two to four crates from each plot were placed in the Montreal Harbour Cold Storage.

MATERIALS AND METHODS

The field experiment was based on a 4-8-16 fertilizer at the rate of one ton per acre.   Nitrogen was supplied in the form of nitrate of soda and potash as the muriate form.   This was accepted as the unit standard. The treatments were varied so that nitrogen, phosphorus and potash had three levels each, *viz.*, zero, once and twice the amount present in the 4-8-16 fertilizer.   These three levels were arranged in all possible combinations, which gave 27 treatments (Table 1).

The celery was placed in the cold storage on October 1, 1937.   Readings were taken on osmotic pressure, pithiness, colour and breakdown, and were taken at the following times: 50, 71, 96, 106, and 120 days after being placed in storage.

De Vries plasmolytic method was used to determine the osmotic pressures. This method was devised in 1884 and is based on the following considerations (13): The higher the osmotic pressure of the external solution above the osmotic pressure of the cell sap, the greater is the shrinkage of the protoplasm. On the other hand, the smaller the decrease in volume of the protoplasmic sac, the less it will withdraw from the cell wall, thus showing a smaller difference between the two pressures. If a concentration of the outer solution is found which causes but an incipient shrinking of the protoplasm, which is usually observed in some corner of the cell, one may then assume that this concentration of the outer solution balances the concentration of the cell sap within. Since the concentration of the outer solution is known, it is easy to calculate its osmotic pressure and from this the almost equal osmotic pressure of the cell sap.

TABLE 1.—FERTILIZER TREATMENTS SHOWING FORMULAE AND ACTIVE INGREDIENTS IN POUNDS PER ACRE

Treatment	Formula	Nitrate of Soda	Superphosphate	Muriate of Potash
0-0-0	0-0-0	0	0	0
0-0-1	0-0-16	0	0	666.6
0-0-2	0-0-32	0	0	1333.3
0-1-0	0-8-0	0	800	0
0-1-1	0-8-16	0	800	666.6
0-1-2	0-8-32	0	800	1333.3
0-2-0	0-16-0	0	1600	0
0-2-1	0-16-16	0	1600	666.6
0-2-2	0-16-32	0	1600	1333.3
1-0-0	4-0-0	533.3	0	0
1-0-1	4-0-16	533.3	0	666.6
1-0-2	4-0-32	533.3	0	1333 3
1-1-0	4-8-0	533.3	800	0
1-1-1	4-8-16	533.3	800	666.6
1-1-2	4-8-32	533.3	800	1333.3
1-2-0	4-16-0	533.3	1600	0
1-2-1	4-16-16	533.3	1600	666.6
1-2-2	4-16-32	533.3	1600	1333.3
2-0-0	8-0-0	1066.6	0	0
2-0-1	8-0-16	1066.6	0	666.6
2-0-2	8-0-32	1066.6	0	1333.3
2-1-0	8-8-0	1066.6	800	0
2-1-1	8-8-16	1066.6	800	666.6
2-1-2	8-8-32	1066.6	800	1333.3
2-2-0	8-16-0	1066.6	1600	0
2-2-1	8-16-16	1066.6	1600	666.6
2-2-2	8-16-32	1066.6	1600	1333.3

The problem then is to find a concentration of the external solution that produces only incipient plasmolysis. For this purpose a series of solutions of increasing concentration was prepared, differing from one another by the same magnitude, for instance, by 0.01 mol. Thin sections of celery were placed in different solutions. The highest concentrations induced strong plasmolysis (Figure 1); the weakest, no plasmolysis whatever (Figure 2). Somewhere in between, a concentration was found which

FIGURE 1. Strongly plasmolysed cells.
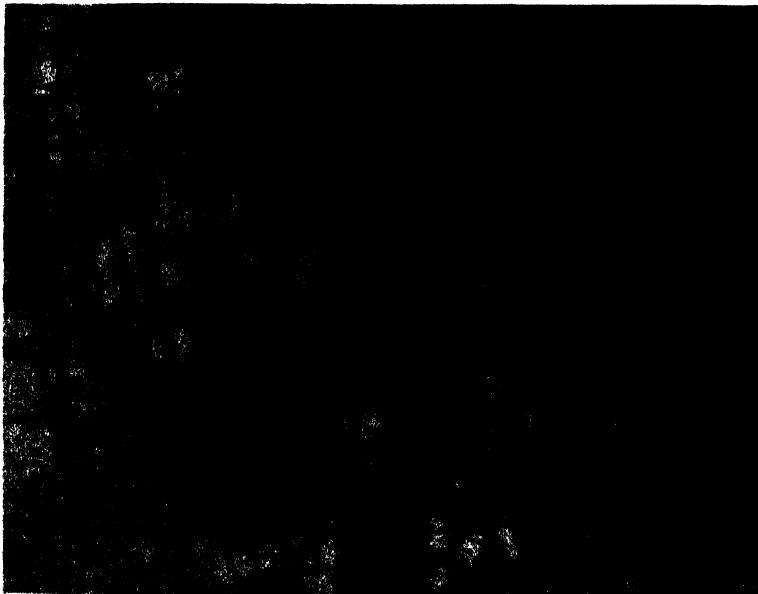
FIGURE 2. Stained cells showing no plasmolysis.

FIGURE 3. Cells showing incipient plasmolysis.

induced only the incipient stage of plasmolysis (Figure 3). The concentration of this solution corresponded to the molecular concentration of the cell sap.

Pithiness was measured by giving a number according to the size of the cavity and the number of stalks showing pithiness. For instance: solid petioles −0, faint trace of pithiness −1, cavity just discernible −2, cavity of fair size −3, and cavity large −4.

Earlier workers, particularly Austin and White (2) required that considerable pithiness be present before the plant was considered pithy. A stalk was not counted pithy unless the whole heart as well as the outer part was pithy. When the three or four outer petioles were somewhat pithy and the heart solid, the plant was counted solid.

In the present work, an attempt was made to differentiate between pithiness and non-pithiness. Indeed, even a single petiole showing only a trace of pithiness was recorded. In this way it was hoped that the pithiness might be determined as being due to treatments, senescence, or position of the petiole on the plant.

Colour was rated according to numerical standards, e.g.: dark green 4, green 3, light green 2, and blanched 1.

Breakdown was determined by weighing before and after trimming off the unmarketable parts. If a part of the petiole was rotted or had become soft and worthless through wilting, only the worthless part was removed, not the whole petiole as would have been done in commercial trimming.

The storage temperature was found to be 32.1 ± 4.06. This was determined by a recording thermometer which operated for a period of about four weeks between November 7 and December 10. It was impossible to determine the relative humidity accurately but it fluctuated between 93% and 97%.

The celery was remarkably free from disease.

REVIEW OF LITERATURE
Osmotic Pressure

Maximov (13) states that in some plants osmotic pressure is induced chiefly by sugars and organic acids. When starch is hydrolysed into sugar, the osmotic pressure is increased considerably.

Lewis and Tuttle (11) found that variation of the sugar content closely followed the variation of the osmotic pressures. They also found that the sugars show a decided concentration during the winter months.

Dixon and Atkins (7) state that the major part of the osmotic pressure of tissues is due to dissolved carbohydrates.

Magness (12) and others have reported a slight increase in the total quantity of sugar in the apple between the time of harvesting and the time when the fruit became soft.

Pithiness

Sandsten and White (17) and Austin and White (2) found that pithiness is hereditary and dates back to the parent plant.

Norton (16) states that pithiness is characterized by a lack of parenchyma, due in self-blanching varieties to heredity, to the propagation of an undesirable strain or to reversion, but in other forms it is probably due to unfavourable cultural conditions.

Early maturity in the field and too high a temperature in the store house cause pithiness, according to Mills (15).

Sayre (18) says that pithiness evidently is correlated with a breaking down of the parenchyma which leaves large open spaces through the centre of the stalk.

Emsweller (8) describes two kinds of pithiness. There is the type which is found throughout all the petioles, even in the young plants, and which is caused by a single dominant gene. The second type develops in the outer petioles with maturity.

Binkley (3) attributes pithiness to growth checks, poorly selected seed, to too rapid a growth following a growth check, and to severe injury by late blight and by web-worm.

EXPERIMENTAL RESULTS

Osmotic Pressure

De Vries plasmolytic method was used. Incipient plasmolysis, the stage when 50% show signs of plasmolysis and 50% do not, was considered as giving a fairly good reading of the osmotic concentration. Maximov (13) shows that the isotonic value at incipient plasmolysis is necessarily a little higher at this stage than normally. However, since this difference is common to all the readings, this method was accepted as giving a fairly good measure of the osmotic pressure. It also has the advantage of being independent of the fluctuation of the water content.

There were no plants of the 2-0-0 treatment after the first of the year since only two crates of this treatment had been placed in storage and they were unfortunately used for another purpose. However the three remaining readings for this treatment were estimated by a method derived by Yates (20) to restore orthogonality and to make the estimate of the error more valid.

Lewis and Tuttle (11) and others claim that there is a rise in the osmotic pressure during the fall and winter seasons until it reaches a certain maximum and then gradually falls off. Some treatments showed a rise, a fall and then a rise to a maximum before the final decline. Others showed an almost steady decline from early in the season. However, when the average of all treatments is considered, a curve is produced which has its maximum about December 10 (Figure 4). This means that the sugar content is at its highest about the tenth of December and then falls off.

Careful study of Table 2 will reveal that there is considerable fluctuation in the osmotic values of the different treatments at different dates. There seems to be little consistency in these data as they stand. However when they are analysed statistically, they show definite results.

The data were analysed by Fisher's Analysis of Variance (9, 10) and the significance tested by the Distribution of z.

The difference in osmotic pressures between the outer and inner petioles was not found to be significant.
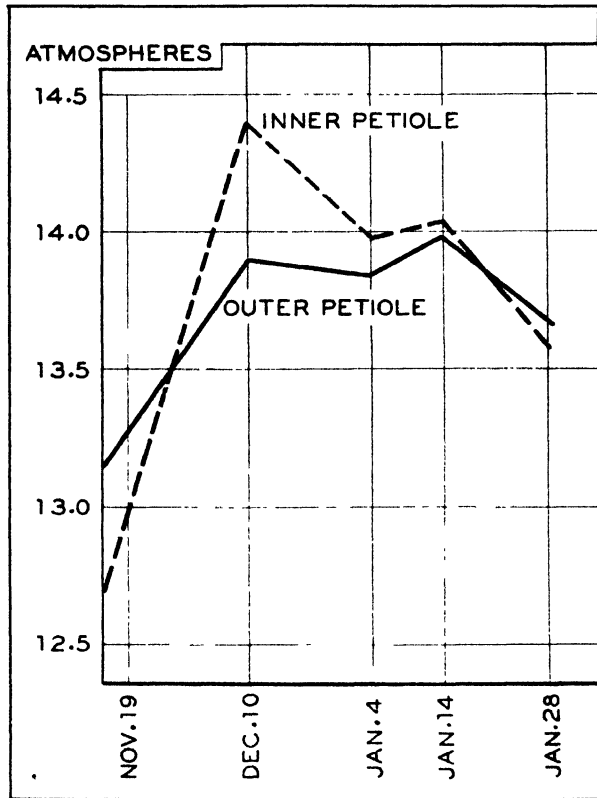
FIGURE 4. Osmotic pressures. Average of the 27 treatments.

Corbett and Thompson (6) have suggested that a progressive translocation of sugars proceeds from the external petioles to the heart during the cold storage of celery.   The present data seem to show that this translocation did not take place in sufficient quantities to be significant.

Length of storage period, of course, is an important factor, as already pointed out.

The results indicate that on the average high nitrogen, low phosphorus and low potash give high osmotic pressures.   However, there are complicating interactions which make it difficult to say which treatment is the best.

Pithiness

The data indicate that the position of the petioles on the plant, whether inner or outer, is highly significant.   The outer petioles showed considerably more pithiness than the inner.   However, it was found that pithiness did not increase with maturity or senescence, as Emsweller's second type of pithiness had done.

Phosphorus is the most important single factor which influences pithiness.   With no phosphorus present, the amount of pithiness was very great.   When 8% and 16% of phosphorus were applied, the amount

TABLE 2.—OSMOTIC VALUES OF INNER AND OUTER PETIOLES OF PLANTS FROM THE DIFFERENT FERTILIZER TREATMENTS AT DIFFERENT DATES

Treatment	Nov. 19		Dec. 10		Jan. 4		Jan. 14		Jan. 28	
	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner	Outer	Inner
0-0-0	.24	.21	.23	.23	.22	.22	.23	.23	.22	.21
0-0-1	.22	.19	.21	.22	.21	.22	.23	.23	.23	.22
0-0-2	.18	.20	.24	.25	.23	.23	.24	.23	.25	.25
0-1-0	.20	.18	.21	.23	.22	.23	.21	.22	.21	.21
0-1-1	.18	.17	.21	.22	.21	.22	.22	.20	.22	.22
0-1-2	.20	.17	.21	.22	.22	.22	.21	.21	.22	.22
0-2-0	.21	.21	.22	.23	.22	.22	.23	.23	.23	.23
0-2-1	.22	.21	.21	.23	.22	.22	.24	.24	.22	.22
0-2-2	.22	.21	.23	.24	.22	.22	.22	.23	.21	.21
1-0-0	.22	.22	.25	.25	.25	.25	.25	.25	.25	.25
1-0-1	.22	.22	.25	.25	.25	.25	.25	25	.24	.24
1-0-2	.21	.20	.20	.21	.23	.22	.21	.21	.20	.20
1-1-0	.20	.21	.24	.25	.24	.25	.24	.24	.23	.23
1-1-1	.21	.20	20	.20	.20	.21	.21	.21	.21	.20
1-1-2	.21	.20	.20	.20	.22	.21	.20	.21	.20	.19
1-2-0	.20	.19	.21	.22	21	.21	.21	.21	.20	.20
1-2-1	.19	.19	.22	.23	.20	.21	.22	.21	.20	.20
1-2-2	.21	.21	.22	.23	.23	.22	.22	.21	.21	.21
2-0-0	.23	.23	.23	.24	.23	.24	.24	.24	.23	.24
2-0-1	.23	.22	.24	.25	.25	.26	.26	.27	.26	.26
2-0-2	.22	.23	.24	.26	.23	.23	.23	.23	.23	.23
2-1-0	.21	.20	.23	.24	.22	.22	.23	.24	.23	.23
2-1-1	21	.21	.22	.23	.24	.24	.24	.24	.23	.23
2-1-2	.21	.22	.22	.22	.20	.20	.20	.21	.18	.19
2-2-0	.23	.22	.24	.25	.25	.25	.24	.24	.23	.24
2-2-1	.22	.19	.24	.25	.21	.21	.22	.22	.23	.23
2-2-2	.23	.22	.23	.24	.21	.21	.20	.21	.19	.19

of pithiness was considerably reduced, being smaller with the 16% than with the 8% but not significantly so. Nitrogen and potash did not show any significant differences.

White-Stevens (19) also found that nitrogen was not effective in causing pithiness.

The interactions of P × K and P × N are highly significant, whereas N × K is just barely significant.

Colour

Colour changes of the celery in cold storage were observed over a period of four months.

Phosphorus, nitrogen and potash are all highly significant though phosphorus is really outstanding.

When the three elements are lacking, the plants maintain quite a deep green colour, throughout the storage period.

The data would indicate that a medium amount of nitrogen and a medium to large amount of phosphorus and potash cause the petioles to blanch best in storage. Plants receiving this treatment would, of course, be large and would blanch in the field to a certain extent due to the petioles being covered by the abundant foliage.

Breakdown

Had the plants been weighed at the time that they were put in storage and again after they had been trimmed after being in storage for a certain length of time, a good idea of the amount of breakdown would have been obtained.   However, the plants were not weighed on being put in the cold store so the author had to be content with weighing them as they came out of storage, both before and after trimming.   These data were used in estimating the percentage of breakdown.

The Analysis of Variance revealed that treatments, phosphorus, and period of storage were all highly significant.

Phosphorus was the most important factor in influencing the amount of breakdown.   The heavier application of phosphorus proved to be the one which gave the least amount of breakdown.   However the difference in favour of the 16% over the 8% was not quite enough to be significant.

Four per cent nitrogen produced more breakdown than either the 0 or the 8% treatments.   The higher percentage of nitrogen proved to be the best.

Potash did not produce significant differences in this respect.

Thus it is seen that a heavy application of nitrogen and a heavy application of phosphorus produced the smallest percentage of breakdown.

DISCUSSION

Abell (1) states that a high nitrogen content of the soil is necessary for good quality of celery.   The data from this experiment are in agreement with this.

Contrary to popular belief, potash does not show up as being of particular importance to celery grown on muck soil, especially from the standpoint of keeping quality in cold storage.   This is in direct variance with the results obtained by F. S. Browne (5) at the Ste. Clothilde Substation last summer.   He found that when the plants received a side-dressing of 200 pounds of muriate of potash during the summer, the yield was substantially increased and the plants kept in much better condition in the storage till the end of December than did the plants which received the same fertilizer treatment except for the side-dressing.   The author is at a loss as to how to reconcile these two differences since the material used by him was grown on muck only a few miles from Ste. Clothilde.

Phosphorus seems to be the most important in connection with keeping quality as determined by osmotic pressure, pithiness, colour and breakdown.   Its level should be accurately adjusted since fluctuation in it in one direction or the other is most important, whereas fluctuations in either nitrogen or potash are not as important.

A number of correlations were calculated.   The figures for yield of these fertilizer plots were taken from Bourque (4), Quebec bursary student working under the supervision of the Working Committee of the Quebec Refrigeration Committee.

The correlation coefficients obtained were tested by Table VA (9).

Thus there are negative correlations between yield and osmotic pressure, yield and colour, and yield and pithiness.   Therefore as the yield

increases, colour and pithiness noticeably decrease, whereas the correlation between yield and osmotic pressure is not very high though it is significant. There seem to be a factor or factors apart from growth which affect osmotic pressures.

From Table 3, it is seen that breakdown, colour and pithiness all increase as the sugar content is increased, though none of the correlations are very high.

TABLE 3

Correlation between	Correlation coefficient	Cor. Coeff. Value P. 02	Cor. Coeff. Value P. 01
Osmotic concentration and breakdown	0 448	0.4451	0.4869
Osmotic concentration and yield	−0.438		
Osmotic concentratio 1 and pithiness	0.509		
Osmotic concentration and colour	0.623	0.4451	0 4869
Yield and colour	−0.804		
Yield and pithiness	−0.602		
Colour and pithiness	0.694		

Plants which maintain their green colour to a greater or lesser extent throughout storage seem to be more pithy than the lots which blanched more completely.

Bourque (4) in analysing the yield results of this experiment found that the 2-1-1 (8-8-16 fertilizer) treatment gave the best results. These present studies seem to indicate that of the 27 fertilizer treatments used in the experiment, the 2-1-1 is probably the best from the standpoint of keeping quality of celery in cold storage.

However, in the face of these physiological studies the 8-12-16 fertilizer might be recommended for trial. These results are based on one year's work and therefore are not altogether conclusive.

SUMMARY

1. Osmotic pressure was measured by De Vries plasmolytic method. The difference between outer and inner petioles was not found to be significant. The osmotic pressures reached a maximum about the tenth of December.

2. Pithiness was observed and recorded. The outer petioles showed more pithiness than the inner. Pithiness did not increase with senescence.

3. Colour was observed over a period of four months. There was quite a marked difference due to treatments.

4. Breakdown was determined. Phosphorus, treatments, and period of storage proved to be highly significant.

5. Phosphorus seems to be the element which requires careful adjustment, since it figures highly in each of the four studies. This will depend, probably, to a considerable extent on the phosphorus content in the soil.

6. Nitrogen fluctuations are not so important though a fairly high level should be maintained.

7. Potash does not seem to be as important as it sometimes is claimed to be, since in none of the four studies did it produce significant differences.

8. Increased yield gave decreased colour and pithiness and to a lesser extent decreased osmotic pressures.

9. Increased osmotic pressures gave increased pithiness, colour and breakdown, though the correlation is not very high.

10. Plants which kept their green colour throughout storage were on the average quite pithy.

11. These studies point to an 8-8-16 fertilizer as being the best of those used in the experiment, though the data might suggest an 8-12-16 fertilizer as being even better.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to those who made this work possible, more especially to Professor H. R. Murray of the Horticultural Department, who suggested the problem and gave the advice and assistance necessary in the field and storage work; to Professor R. Summerby of the Agronomy Department, for his instruction regarding statistical analysis; to Dr. G. W. Scarth of the Botany Department, McGill University, for his instruction regarding osmotic pressures; and to Mr. P. Bertrand, who assisted in taking notes on pithiness, colour and breakdown.

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THE APPLICATION OF THE CONTROLLED ATMOSPHERES IN THE STORAGE OF FRUITS

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THE APPLICATION OF CONTROLLED ATMOSPHERES IN THE STORAGE OF FRUITS[1]

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It is apparent from observation that fruit suffering from physiological storage disorders is being marketed to quite a large extent. It is not uncommon to see apples on display during the winter suffering from superficial scald and similar disorders. In many cases, McIntosh apples on the market are being sold as high quality apples, but on cutting these open they are found to be suffering from core flush and other forms of internal breakdown. As long as these conditions are allowed to exist the demand for home grown fruits is bound to decrease.

The situation becomes more grave when we consider the probable increase in apple production in the next few years. According to surveys made by the Quebec Pomological Society, out of the 600,000 commercially grown apple trees in Quebec, 326,000 are non-bearing. Out of these non-bearing trees about 60% are McIntosh variety. If these figures are representative of Canada as a whole it is obvious that the trend of production is swinging to an excess, especially in the case of McIntosh.

One of the unfortunate features of this variety is that it holds its appearance sufficiently well in storage to permit marketing after the natural flavours have disappeared and the quality is low. Another point is that McIntosh, if stored at a temperature of 32° F. to obtain maximum storage life, develops core flush.

Core flush is definitely associated with low storage temperatures in the case of McIntosh apples. If core flush development is controlled by using a higher storage temperature a considerable loss in storage life results.

This is the point where a system of controlled atmospheres has proven to be of extreme value. With McIntosh apples an atmosphere of 7% CO_2 and 14% O_2 at 39° F. will extend the storage life to the same extent as 32° F. without the development of core flush. Thus the advantage in improving the marketing condition of McIntosh apples can readily be seen. Similarly the effect of gas storage (or controlled atmospheres) has been shown to be beneficial in the storage of other varieties of apples and pears. There is no reason to believe that it will not have some application in vegetables and other horticultural produce.

Controlled atmospheres in storage, as the name implies, is merely a control of the concentration of atmospheric gases. When fruits are stored in a gas tight chamber, the concentration of oxygen is reduced and carbon dioxide increased by the respiratory activity of the fruit. In order that the concentrations of these gases shall not reach a point where damage is caused to the fruit tissues, some control system must be devised. In most cases a simple ventilation system is all that is necessary. When subnormal amounts of oxygen are required a CO_2 absorber is used.

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  Contribution No. 517 from the Division of Horticulture, Dominion Experimental Farms System.
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This brings up the problem of balance between CO_2 and O_2 concentrations. It has been shown that lowering the concentration of oxygen decreases the respiratory activity. Furthermore, the lowered oxygen concentrations affect the respiratory reactions previous to glycolysis (Blackman—1928). This applies, of course, while the tissues are respiring aerobically.

Investigatory work carried out with the effect of controlled atmospheres on Bartlett pears has indicated that a balance between CO_2 concentration and oxygen concentrations should exist. It is then feasible to assume that if CO_2 acts as a depressant it would be in the form of a post-glycolytric narcotic. This is substantiated by the trend of respiratory quotients, particularly at the onset of gas storage injury. Be this as it may, the balance between oxygen decrease and CO_2 increase has been found to be important. Fortunately in most cases Canadian grown apples do well under a balance brought out by the fruit themselves. Probably the only important exception is in Cox Orange variety of apples which requires a sub-normal oxygen mixture.

The next feature to be considered is absolute concentrations. The direct ill effect of concentrations is brought about by inducing a state of zymasis. In other words, the oxidative system must perform in such a manner that the substrates for respiration are completely oxidized. Partial oxidation results in the production and subsequent accumulation of such products as ethyl alcohol and acetaldehyde. If this state of metabolism exists for any length of time, necrosis of the tissues is the ultimate result. The physiological disorder resulting from these conditions has been referred to as brown heart. In many cases, however, zymasis may be induced only to a slight extent resulting in lack of quality and poor flavours. This upset in metabolism is the main consideration in the application of controlled atmosphere. The criteria used to foretell these circumstances are changes in respiratory quotient and detection of mal-flavours. We have resorted chiefly to the latter on the bases of reliability and convenience.

Thus the chief problem in the application of controlled atmosphere is presented *viz.:* to arrive at a controlled atmosphere which will produce maximum prolongation of storage life without a deleterious upset to the metabolism.

Our method of working on this problem is to expose fruit in small chambers to a continuous flow of gas of a given CO_2 and O_2 concentration (the balance being made up of nitrogen). These atmospheres are made up in a gas mixing chamber and compressed into a gas cylinder. A flow of 1 cu. ft. per day per chamber is permitted to pass over the fruit from these cylinders.

Examinations of the fruit made after removal from the chambers and being exposed to ripening conditions for one week have revealed some interesting facts, some of which have been indicated by other workers. In brief, the most salient of these are: (1) different varieties require different concentrations; (2) the same variety may require a different gas concentration at different temperatures; (3) different maturities of the same variety behave differently under the same gas mixtures at the same temperature.

As has been suggested, McIntosh is probably the most important variety (from the standpoint of results and commercial importance) and hence work has been concentrated on this particular variety. It was found that the best results, as mentioned at the outset, were obtained by exposing this variety to a $7 \pm \frac{1}{2}\%$ CO_2 and $14 \pm \frac{1}{2}\%$ O_2 at 39° F.

The next step was to reproduce these conditions in a semi-commercial way. This was carried out in a room holding about 150 bushels. These apples were marketed on the local market during the winter and comparisons were made with ordinary cold stored apples.

The results in brief from this latter trial indicate that McIntosh apples stored under these gas storage conditions were slightly superior in quality to the same apples stored at 32° F. and were slightly less advanced in maturity according to pressure tests. Humidity conditions being much better controlled, the fruit was more turgid and crisp. The most important feature was that no core flush developed in the gas stored apples whereas amounts varying from 20% to 25% were found at 32° F. normal storage (1936–37 Report).

Since such encouraging results were obtained by the use of a controlled atmosphere with McIntosh apples, a more minute investigation into the effect of apple type on subsequent quality of the fruit was made during the past year. The results obtained indicate that for maximum quality, well blushed fruit, well matured and of medium large size ($2\frac{1}{2}''$–$3''$) should be used. Early picked small fruit failed to develop maximum quality and aroma in storage.

It is hoped that by autumn of 1938 at least one commercial gas storage plant will be in operation for the storage of McIntosh apples. If this proves successful, the marketing situation of McIntosh should be improved, making it possible to offer fruit for sale which is not internally diseased and which is otherwise improved in quality. One feature must be emphasized and that is that controlled atmospheres should not be used for prolonging the storage life of McIntosh. At the present time McIntosh are being sold far beyond the end of their normal storage life. The chief aim should be to market McIntosh apples before the end of January at their highest standard quality. The logical and feasible method for doing this is by applying controlled atmospheric conditions judiciously.

THE EFFECT OF EXPOSURE OF FRESH FILLETS TO ULTRAVIOLET LIGHT ON THEIR SUBSEQUENT KEEPING QUALITY

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In view of the prohibitions regarding the use of chemical preservatives in fish and fish products, experiments were undertaken at this Station in order to ascertain whether or not other acceptable means of exerting a favourable effect on the keeping quality of fresh fillets might be developed. Since the fundamental discovery of Downes and Blount in 1877 that ultraviolet light (U.V.L.) is lethal to micro-organisms, researches carried out in more recent times have shown that rays having a wave length of from about 2500 to 3000 Angstrom units are the most bactericidal — depending, apparently, upon what type of micro-organism is being dealt with. The possibilities surrounding the application of such rays to the sterilization or partial sterilization of foodstuffs were foreseen many years ago, but unfortunately, their relatively feeble penetrating power severely restricted their field of usefulness. Recently, there has been developed a commercial ultraviolet lamp which, it is claimed, produces 80 to 85% of its emanations in the most bactericidal part of the spectrum — namely between 2800 and 3000 Angstrom units. Startling results are said to have been obtained with such lamps in retarding the growth of micro-organisms during the storage and "ripening" of meat; but it is fairly well known that the heaviest contamination invariably occurs on the surface of meat and thus such rays would be expected to exert a marked effect.

So far, few if any experiments appear to have been made in order to ascertain whether U.V.L. might be used with advantage to enhance the keeping quality of fish or fish products, and work was therefore initiated in this direction. A wooden cabinet was constructed which had external dimensions of 2 x 5 ft. by 4 ft. high and was entirely lined with bright tin so shaped that reflection would tend to make the intensity of radiation fairly constant throughout. The cabinet was portable and self-contained, two of the quartz tube lamps 30 in. long being installed vertically at its ends. The fillets to be treated were exposed by hanging them from 3 to 6 in. apart along a rod which was fixed 6 in. from the the ceiling. These lamps do not cause any lasting harm to the operators, but a slight superficial "sunburn" will result from prolonged exposure, and great care must be taken to protect the eyes by use of either ordinary or lightly-coloured glasses, otherwise very painful effects will be experienced.

It is well known that U.V.L. has rather meagre penetrating power with respect to animal tissues and, therefore, all experiments have been designed so that the fillets exposed to them should have a maximum surface contamination. To this end experiments have in most cases been made with very recently caught fish, for in these the muscle itself will be bacteriologically sterile, or nearly so, and the contamination which occurs during the filleting procedure will probably be largely on the surfaces of the fillets. In many instances small experimental fillets were used; but in some, large fillets such as might be met with commercially were employed. The technique followed varied somewhat in

different experiments but the general procedure was to fillet the fish in the customary manner, to expose one half the fillets to U.V.L. for a given length of time, and to use the remainder as controls. In some experiments the fish were filleted in the presence of U.V.L., the fillets obtained being then further exposed for a short time. In such cases the controls consisted of fillets of fish filleted under similar conditions in the absence of U.V.L. The fillets were stored at approximately 35°F. either in large sterilized glass-covered dishes, or wrapped in moisture-proof cellophane. It is not considered necessary to explain here the methods employed for sampling the fillets for bacterial counts; brief reference has already been made to the general technique followed in a recent article by Tarr and Sunderland in Progress Report No. 37. The fillets used in the following experiments were all skinned.

Experiment 1 — Four fillets about 6 x 1⅜ x ⅜ inches in size were made from a chicken halibut that had been stored for 3 days in ice. These were exposed to U.V.L. after the ends had first been snipped off with sterile scissors. Samples were taken at intervals by snipping off about ½-in. pieces from each fillet and pooling the muscle obtained for bacterial counts:

Exposure in minutes:	0	5	10	15	20
*Bacterial count:	35,200	22,760	17,840	25,120	16,780

*In this and all subsequent tables these figures are given as counts (colonies) of viable organisms (still capable of growth) per gram of muscle.

Experiment 2 — Eight fillets of about the same size as used in experiment 1 were made from a cod which had just been caught. These were sampled as in experiment 1 before and after 35 minutes exposure to U.V.L. with the following result:

Bacterial count before exposure	420,000
Bacterial count after 35 min. exposure to U.V.L.	75,000

Experiment 3 — Four fillets of cod from a recently caught fish were made as in experiment 2 and samples were taken throughout a 24-hour period as follows :

Exposure to U.V.L.	Bacterial count
0 minutes (control)	172,800
40 minutes	78,800
4 hours	64,000
7 hours	156,000
24 hours	11,820,000

Experiment 4 — Twelve fillets of about the same size as those used in the previous experiments were prepared from different species of so-called flounders caught only a few hours previously. Six of these selected at random were exposed for 30 minutes to U.V.L., and the remainder were untreated. The two lots were stored separately in sterilized covered glass dishes for 7 days at 35°F., and were then tested bacteriologically and organoleptically with the results shown below:

Bacterial counts:	Controls (unexposed)	2,042,000,000
	Exposed 30 min. to U.V.L.	606,000,000

Organoleptic tests: Four individuals declared that the untreated fillets were extremely stale or putrid in odour, while th treated ones were not nearly so objectionable, or only slightly stale. On cooking the fillets, four out of five persons stated that they definitely preferred the treated ones, only one person preferring the untreated

Experiment 5 — Two gray cod about 4 to 5 ℔ in weight held in ice for 3 days were used. One of these was filleted as usual while the other was filleted under similar conditions except that the whole process was carried out in the presence of U.V.L. In each case the fillets were wrapped in moisture-proof cellophane, which was also exposed to U.V.L. in the case of the treated fish, and stored for 10 days at about 35°F.

Bacterial counts. Unfortunately these are only very approximate since adequate dilutions were not made:

Controls	7,200,000
Exposed to U.V.L. during filleting	3,600,000

Organoleptic tests: Three tasters definitely preferred the treated fish as regards odour when uncooked and flavour subsequent to cooking. Neither lot of fillets was in excellent condition; but whereas the treated fish were edible though by no means strictly fresh, the untreated fillets were distinctly sour and not fit for food.

Experiment 6 — A ling cod of about 28 ℔ weight, that had been held in ice for 2 days, was filleted and two of the large fillets were each cut in four pieces. Four of the eight fillets thus obtained were selected at random and exposed to U.V.L. for 10 minutes, the remaining four being untreated. The fillets were wrapped in cellophane and stored for 10 days at approximately 35°F. with the following results:

Bacterial counts:	Controls	19,800,000
	Exposed 10 min. to U.V.L.	19,600,000

Organoleptic tests: Three tasters could not decide definitely which fillets were freshest as regards uncooked odour, though there appeared to be a slight bias in favour of the treated fish. After cooking the fillets, two preferred, but only very slightly, the treated fish; the other taster declared no significant difference could be detected.

Experiment 7 — Two halibut iced for three days and weighing approximately 7 ℔ were filleted, one under U.V.L. and the other under similar conditions but in the absence of U.V.L. The fillets from the treated fish were given an additional 5 minutes exposure to U. V. L. subsequent to filleting. The treated and untreated fillets were wrapped in moisture-proof cellophane, stored at about 35°F. for 10 days, and then left for 16 hours at room temperature before examining:

Bacterial counts:	Controls	17,180,000
	Filleted under and exposed	
	for 5 min. to U.V.L.	2,320,000

Organoleptic tests: Three tasters found that the odour of the uncooked control fish was slightly stronger than that of the treated fish, but that after cooking no difference in flavour was noticeable.

Discussion

The above experiments must be regarded as strictly preliminary and of a exploratory nature, but are typical ones selected from a fairly large number. Though the results obtained have not in all cases demonstrated a consistent and clear cut improvement in keeping quality following exposure of fresh fish fillets to U.V.L., there is a very strong tendency for fillets thus treated to have lower bacterial counts and better flavour and odour on keeping than have untreated fillets. That the rays act only superficially is apparent, for bacterial multiplication proceeds actively even in exposed fillets, as can be seen from experiment 3. Such results are fairly obviously due to the fact that the rays penetrate only

slightly, and that the organisms in the non-exposed or protected interior portions of the fillets multiply actively. The minimum effective time of exposure required to establish a maximum decrease in bacterial population has not yet been definitely established, but from the results of experiment 1 it would appear that 5 or 10 minutes is effective. It is not improbable that pigmented fish may absorb the rays preferentially or that in coarse fish muscle the bacteria may sink into the flesh and be protected from the light. These and other factors remain to be investigated. There are other possible uses for U.V.L., one of which is the use of such rays in reducing the bacterial population in brines and pickles in which fillets are frequently immersed, and which serve as a serious source of contamination. It is intended to study this and other possible uses of U.V.L. in attempting to improve the quality of fish or fish products.

COLOUR OF MEAT: I. APPARATUS FOR ITS MEASUREMENT, AND RELATION BETWEEN pH AND COLOUR[1]

By C. A. Winkler[2]

Abstract

A photoelectric colour comparator, similar to that designed by Bolton and Williams (1), has been constructed and used to compare the colours of meat samples at different pH. Light falls at an angle of 45° on the surface of the sample, and the amount scattered at right angles from the surface in the red, green, and blue regions of the spectrum, defined by standard colour filters in the path of the scattered light, is measured photoelectrically as a percentage of the amount similarly scattered in the same spectral regions from a standard white surface under the same light intensity. The precision of the measurements on meats was $\pm 0.25\%$ scatter with any one of the three filters.

When samples of pork, beef, and mutton were used, after adjustment of the pH by injections of lactic acid or ammonia, the relation between pH and colour was found to be similar for the three meats, with maximum scatter of red, green, and blue at pH about 5.0 – 5.5. When uninjected samples of pork were used, scatter in the three spectral regions decreased over the pH range 5.4 – 6.6, paralleling the changes observed with injected samples within the same pH limits. The visual appearance of the meats is greyish at pH levels acid to the region of maximum scatter and pink in the region of maximum scatter, shading to dark red at higher pH levels. Darkening is paralleled by a decrease in the scatter, while changes in the quality of the colour are accompanied by changes in the ratio of red/green and red/blue.

Introduction

Good colour in meat, while it might not affect its palatability or nutritive value, is generally recognized and demanded by the consumer. The economic significance of colour and colour stability in meat is therefore obvious.

Of the work that has been done on the colour of meat, that of Brooks (2, 3), Mackintosh and his associates (5, 6, 7), and Heiss and Hohler (4) is probably of greatest interest to the meat packing industry. Much remains to be done, however, and studies of the factors that influence the colour and colour stability of meats, particularly pork and bacon, are being made as part of a program of researches on meats in these laboratories.

Sair and Cook (8, 9) have recently published the results of a study of "drip" from various meats. In the course of their work they observed what appeared to be a relation between pH and colour of the meats. The present paper describes a quantitative study of the relation between pH and colour for pork, beef and mutton.

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Apparatus

The colour comparator used in these investigations was similar in essential features to that described by Bolton and Williams (1) and is shown diagrammatically in Fig. 1.
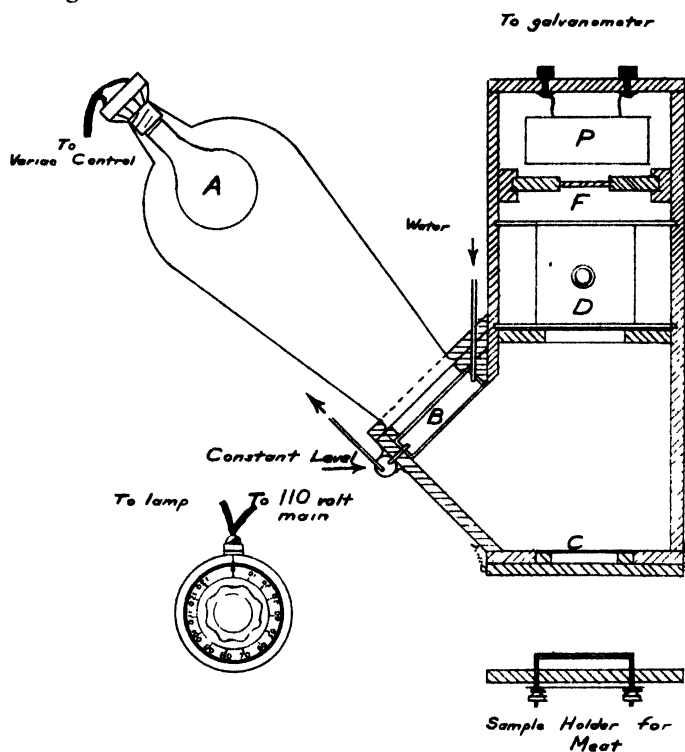
FIG. 1. *Diagram of colour comparator for meats.*

The light source *A* ("Photoflood" bulb) was mounted in front of a reflector and controlled with a "Variac" transformer. The infra-red radiation reaching the sample was reduced by the passage of the light through water in the glass cell *B*. The light fell at an angle of 45° on the surface of the sample at *C*. The light, scattered approximately at right angles to the surface of the sample, passed through another glass cell *D*, containing a dilute solution of copper sulphate, to remove any infra-red that might be present in the scattered light. Suitable glass colour filters were inserted in the path of the scattered light at *F*, and the filtered radiation was received on a "Photronic" photoelectric cell *P* connected to a sensitive galvanometer.

A magnesium carbonate block was mounted on a holder that could be put in place at *C*. A circular area of two square inches of white surface, covered with a thin glass slide, was exposed. The holder for the test sample consisted of a solid back against which the meat, between thin glass slides, was held in position by a stiff metal plate having a circular hole two square inches in area. The area of meat surface and the area of standard white surface exposed

to the light were therefore equal. Small surface irregularities in the meat sample were eliminated by compressing it slightly between the glass slides.

The method used to measure the colour characteristics of a sample of meat was similar to that described by Bolton and Williams (1). With one of the colour filters and the standard white surface in place, the intensity of the light source was adjusted to give some chosen galvanometer deflection d. The standard white surface was then replaced by the meat sample, and the galvanometer deflection d_1 obtained. For the colour filter used, the percentage scatter by the sample is then given by $100 \dfrac{d_1}{d}$. The procedure was repeated for other colour filters.

In preliminary tests with the apparatus, several colour filters were used, with a view to obtaining a fairly detailed spectrophotometric analysis of the meat samples. It soon became apparent, however, that sampling errors were too large to justify a detailed colour analysis, and three filters only were finally used. The wave-length ranges over which these transmitted were: blue, 4000 – 4500 Å; green, 4900 – 5800 Å; red, 5750 – 7000 Å. Results are expressed simply as percentage scatter of blue, green and red.

Tests with the apparatus showed that different "photoflood" bulbs and different photoelectric cells gave the same values for the scatter from a given meat sample. It was also found that the time during which the meat was exposed to the intense light, in making measurements with three filters, was not long enough for the colour of the meat to change under the action of the light. With practice, it was possible to make measurements with three filters in about three minutes, and most of this time was taken up in adjusting the light intensity with the standard white surface in position.

The precision of the apparatus has been determined by successive measurements with a given sample of meat, and by measuring the colour of the surfaces along which the knife passes when two samples are prepared from a single piece of meat. By both these methods the value obtained for the percentage scatter could be checked within $\pm 0.25\%$ with any one of the three filters.

The percentage scatter from two pieces cut from the same muscle of a carcass and brought to the same pH may differ by an amount several times the limit of precision for the apparatus. The magnitude of this sampling error is illustrated in Table I.

TABLE I

VARIATION IN COLOUR, AT THE SAME PH, FOR DUPLICATE SAMPLES FROM THE SAME MUSCLE

Type of meat	pH	Light scattered, %		
		Red	Green	Blue
Pork	5 0	⎰ 21 1 ⎱ 26 2	19 5 23 5	16.8 18.2
Beef	5 5	⎰ 30 0 ⎱ 30 0	14 0 14 4	14.0 14 8
Pork	5 6	⎰ 33 0 ⎱ 31 8	25 4 24 7	19 8 20 0
Pork	5 8	⎰ 39.5 ⎱ 42 4	28 2 26 8	24 3 23.5

The values, taken at random from the results obtained with pork and beef, show at once that the precision of the method adopted for measuring the colour was adequate for the investigation.

Experimental Procedure and Results

Unminced samples (100 to 200 gm. each) from a given muscle of pork and mutton were brought to various pH levels by the method described by Sair
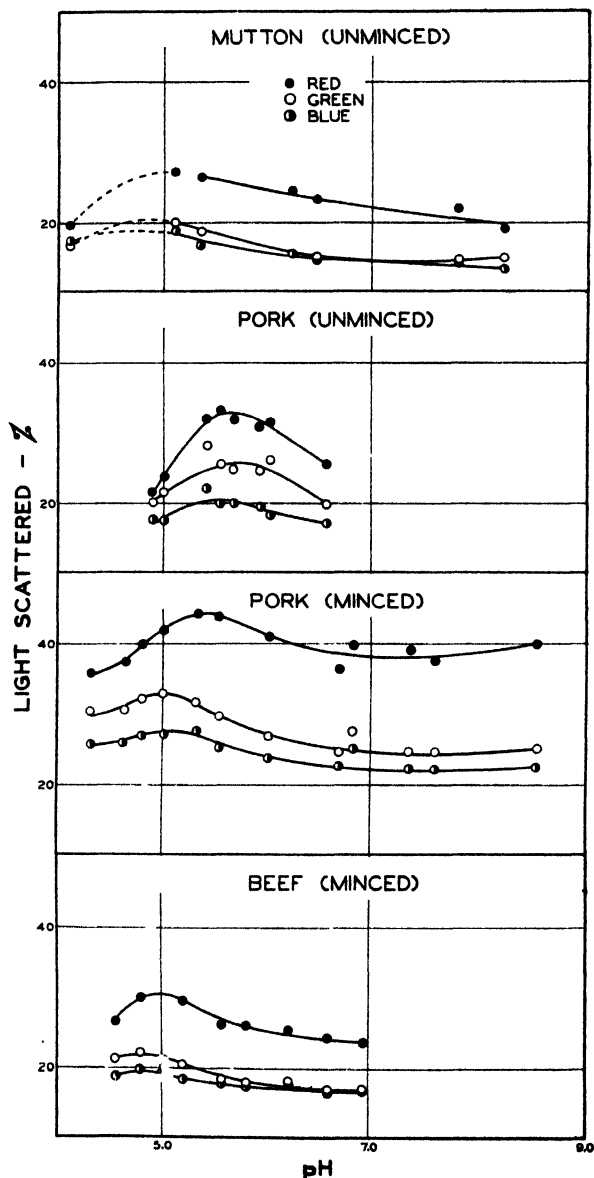
FIG. 2. *pH—Colour relation for mutton, pork and beef. Samples injected with lactic acid and ammonia to alter pH.*

and Cook (9). It consisted simply of injecting into the meat dilute lactic acid or ammonia solutions of suitable concentrations, followed by 3 to 5 days' storage of the samples at 0° C. A slice was taken from each sample after its removal from storage, and the colour measured. The pH of each slice was determined with a glass electrode. A typical curve for pork is shown in Fig. 2; only one experiment was made with mutton, the results of which are also shown in Fig. 2.

The colour, prior to injection, of the samples used in the experiments described above differed appreciably, and it is probable that the variation in initial colour was reflected, to some extent at least, in the colours developed after injection. It was thought worth while, therefore, to check the results by making experiments in which differences in initial colour were eliminated. This was done by mincing and thoroughly mixing the meat before injections were made. The batch was divided into several portions, and their similarity of colour checked with the colour comparator. They were then injected and stored, with frequent mixing. Typical results for minced pork and beef are represented in Fig. 2.

The range of pH obtainable with the injection method is much greater than that existing normally in carcasses. The pH and colour of cuts from a number of hog carcasses were therefore measured to find out if any relation between colour and pH could be detected for untreated pork. The samples were taken from carcasses in a commercial abattoir, the only factor limiting their choice being that they should be from corresponding muscles of the carcasses. Samples were taken on two different days, at least four cuts at each pH being measured. The average values for the colour at different pH levels are plotted in Fig. 3.

In Fig. 4 are plotted the ratios of the scatters together with the scatter of red from the minced pork. The visual appearance of the samples is also given for the pH regions in which the colour changes were sufficiently marked for

Fig. 3. *Average pH—Colour relation for pork from different carcasses. No injections of lactic acid or ammonia. Identity of curves as in Fig. 2.*

recognition; above and below the pH levels at which the meat appeared pink are regions in which it was difficult to describe the colour as it shaded from pink through light brown to grey, or from pink to darker red.

Interpretation and Discussion

By examination of the curve for red scatter (Fig. 4) in conjunction with the curves for the ratios, changes in intensity and quality of the colour respectively

can be fairly well described. As a scattering surface becomes lighter in colour, the percentage scatter of all wave bands increases, while if the surface becomes darker the reverse is true. As the trends of the curves for red, green and blue scatters are similar (Fig. 3) the curve for red scatter alone is sufficient to show that from pH about 4.5 to pH about 5.5 the samples become lighter, and beyond pH 5.5, darker in colour. Fig. 4 shows also that there is an increase of approximately 25% in the ratio of red scatter to blue and green scatters
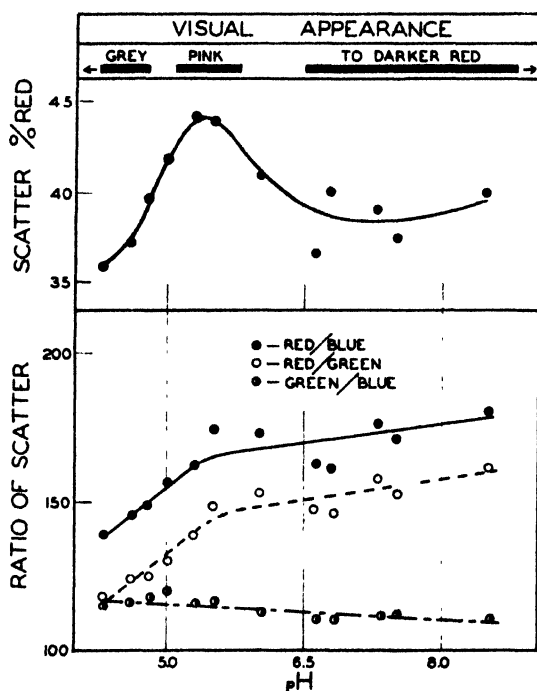
Fig. 4. *Relation between pH and ratios of scatters, red scatter and visual appearance (minced pork).*

over the pH range about 4.5 to 5.5, paralleling the visually observed change in composition of the colour from grey through light brown to the normal pink colour. From pH 5.5 to 8.5 the ratios of red/green and red/blue increase by only about 8%, and the change over this pH range is mainly in intensity, rather than composition, of the colour. The ratio of green/blue (Fig. 4) is almost constant over the entire range of pH, and is therefore of little or no value in determining the nature of the colour changes.

It is apparent from the curves in Fig. 2 that for meat from a given muscle there is a relation between pH and colour of pork, beef and mutton. The general shape of the pH-colour curves is the same for the three meats, and although it is not evident from Fig. 2, other experiments with pork and beef that gave curves quite similar to those shown, indicate that the pH for maximum scatter from these two meats varies between 5.0 and 5.5. There seems,

then, to be no essential difference in the pH-colour relations for pork and beef, and it is not unlikely that the similarity applies also to mutton, although this should not be definitely concluded from the single experiment.

Four curves for unminced pork, similar to the one shown in Fig. 2, were obtained with samples from the corresponding leg muscles of different animals. The data showed that the amount of scatter at the same pH may differ for different animals by an amount comparable with the change in scatter brought about by alteration of pH in material from a single animal over the entire range investigated. It is therefore evident that factors other than pH are equally or possibly more important in determining the colour of fresh meat from a given animal. Nevertheless, if average values for a sufficient number of samples at each pH are plotted, as in Fig. 3, the relation between pH and colour can be observed with material from different animals.

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TENDERNESS OF MEAT: I. A RECORDING APPARATUS FOR ITS ESTIMATION, AND RELATION BETWEEN pH AND TENDERNESS[1]

By C. A. Winkler[2]

Abstract

A recording apparatus for estimating tenderness of meat is described. Samples of approximately equal cross section are cut between blunt jaws brought together by a constantly increasing force. The movement of the jaws is recorded on a drum revolving at constant rate, producing a curve on which the co-ordinates of any point can be interpreted in terms of the thickness of sample cut by a given force. By measuring the areas beneath the curves, and applying a correction for variations in initial thickness of the samples, comparative values for the work required to cut the samples can be obtained. The standard deviation of the mean of duplicate determinations on a single sample is $\pm 3\%$.

The relation between pH and tenderness was investigated by using samples of pork from three animals and adjusting the pH by injections of lactic acid or ammonia solutions. Toughness was at a maximum at pH about 5 0–6 0; at higher or lower pH levels the meat became progressively more tender. Studies with beef gave similar results, but there was some indication that maximum toughness occurs at a somewhat lower pH. Between different animals the pH at which maximum toughness occurred was more variable in beef than in pork.

Introduction

As tenderness is one of the most important characteristics of good quality meat, it is being studied in these laboratories as part of the investigations into the storage and transport of meat. Of the factors affecting tenderness, those that have received the most attention from other workers are: position in carcass (2, 4, 5, 8), collagen content (2–5), freezing (2, 8, 9) and storage (1, 2, 6, 9). Both subjective and objective methods have been used for estimating tenderness, and arguments have been advanced in support of both procedures (6, 8). For routine measurements. however, an objective method is almost essential, owing to the difficulty of establishing a panel of suitable judges that would be available as required. An apparatus combining simplicity of design (2) with the advantages of a recording device (10) has therefore been constructed for estimating tenderness.

Apparatus and Method

A diagram of the recording apparatus is shown in Fig. 1. The sample of meat is placed between the fixed jaw *A* and the movable jaw *B*. The latter is attached to the duralumin lever *C*, which is counterbalanced by the weight *W*. The upper jaw is held by two metal plates between which the lower jaw moves with about 1 mm. clearance on each side. When the slide *F* is pushed to one side in its groove, fine lead shot runs from the hopper *D* into the aluminium pan *E*. A round hole in the slide controls the rate of flow

of the shot. As the weight of the shot is applied to the lever, the movable jaw approaches the fixed jaw, crushing the meat between them. At the same time, the pen G attached to the lever moves downward over a piece of graph paper fastened around the three-inch diameter drum H. The drum is geared to a "telechron" motor, which is started simultaneously with the flow of lead shot. This is accomplished by attaching the switch K to the slide F so that when the slide is opened the switch is closed, and *vice versa*.
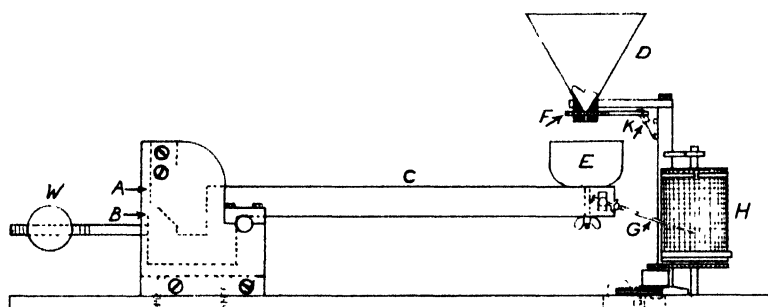
Fig. 1. *Diagram of apparatus.*

The jaws are similar to those described by Volodkevich (10), and are somewhat blunt, rather than sharp. Their action in severing the sample is presumably similar to that of teeth making a single bite.

To obtain curves convenient for subsequent analysis, the speed of rotation of the recorder drum and the rate of flow of lead shot from the hopper are adjusted to meet the requirements of the material being studied. For beef, pork and bacon it has been found satisfactory to use a rate of flow of shot of about 35 gm. per second, and a drum speed of about one revolution in four minutes. The rate of flow of the shot is constant within ± 0.5 gm. per second. With lubrication of the bearing by which the lever is mounted, and accurate adjustment of the counterweight, a weight of 3 to 5 gm. is sufficient to move the lever from a resting position. The errors from variation in rate of flow of the shot and from starting friction are negligible in comparison with sampling errors.

The sample of meat to be tested is trimmed to a length of about 4 cm., a thickness of approximately 1 cm. and such a width (about 1.5 cm.) that it fits snugly between the plates on either side of the jaws. Experiments have shown that the width can be 2 to 3 mm. less than the distance between the limiting plates without detectable error, probably because the meat is spread against the plates almost as soon as the cutting action of the jaws begins. The sample is so prepared and inserted between the jaws that it is cut transversely to the direction in which the fibre bundles lie, *i.e.*, across the "grain" of the meat. Care is taken to avoid obvious striations of connective tissue.

As the weight of the lead shot on the lever increases, and the sample is crushed between the jaws, the pen moves downward over the chart on the

revolving drum to trace a curve similar to those shown in Fig. 2.   Each pair of curves in the figure represent duplicate determinations on samples of meat from the same muscle of pork or beef.   The co-ordinates of a point on such a curve are determined by the amount the pen has moved downward, *i.e.*, by the amount the lower jaw has approached the fixed jaw, and by the length of time the drum has revolved, from which the force acting to cut the sample can be calculated.   Duplicate curves for each sample are started at a common point (*a*, Fig. 2), by adjusting the initial position of the pen.   The end points (*b*, Fig. 2) of the curves, *i.e.*, when the jaws are together, will therefore differ by the amount the pen was adjusted to have the curves start at the same point,
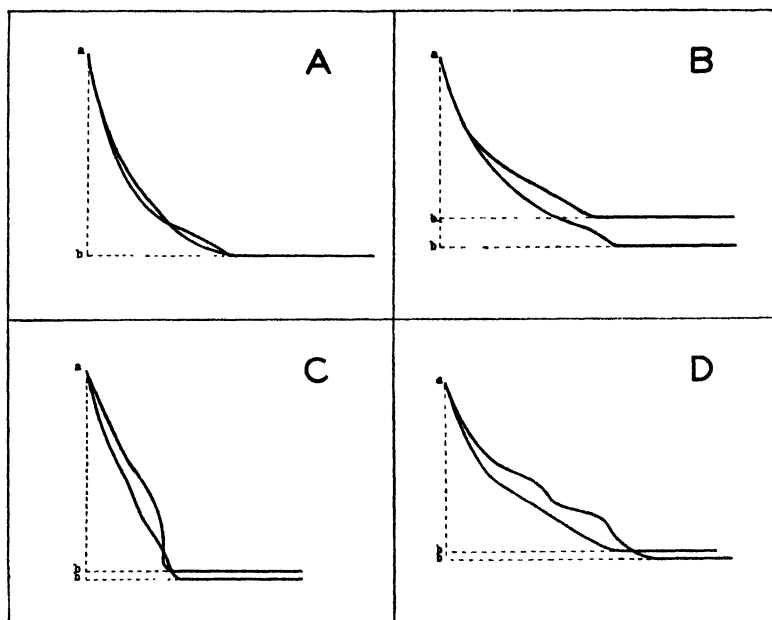
FIG. 2.   *Typical curves obtained with the apparatus.*

and the difference between the end points is therefore a measure of the difference in initial thickness of the samples.   For example, in *A*, Fig. 2, the duplicate samples were the same thickness, while in *B*, *C*, and *D* they were of different thickness.   With the present apparatus there is a slight residual slope at the end point due to flex in the apparatus, but the residual slope is easily identified and seldom interferes with determination of the true end point.   If, as with some samples, the curve approaches the end point very gradually, the constant residual slope can be extrapolated back to the point of intersection with the curve in order to obtain the true end point.   This is seldom necessary as the error in determining the end point is negligible in comparison with sampling errors.

When the constants of the apparatus are known, the force necessary at the jaws to cut through the sample of meat placed between them can be

calculated. The necessary constants are the rate of flow of lead shot into the aluminium pan on the lever, the rate of revolution of the drum carrying the chart, and the ratio of the lengths of the lever arms. For most investigations, however, comparative values of the cutting force are adequate, and the method adopted for expressing the results has therefore been to estimate, with a planimeter, the area in square inches between the curve and two lines, one drawn along the ordinate axis from the starting point, the other drawn along the abscissa axis at the end point. For a given curve, this area will represent the work done in cutting through a sample, the thickness of which is represented by the ordinate distance from the starting point to the end point. To compare different curves on the basis of work done, all work values must be expressed for unit thickness of sample. Let area A under the curve for a given sample correspond to an ordinate distance d (representing the thickness of the sample), and assume that d_1 is the arbitrarily selected ordinate distance representing unit thickness of sample for which the work is to be calculated. Then the work done in cutting unit thickness of the given sample will be $\dfrac{d_1}{d}$. A.

Experiments have demonstrated the validity of this method of correcting all work values to correspond to constant thickness of sample, providing the differences in thickness are not too great. The standard deviation of the mean of duplicate determinations calculated from 20 pairs having mean values varying from 0.8 to 4.6 was $\pm$ 0.05 for samples carefully selected to be free from connective tissue, and varying in thickness by not more than $\pm$ 20%. With some practice it is not difficult to cut the samples to a thickness within about half this tolerance. The precision with which tenderness may be estimated by the method is well within the limits of accuracy imposed by sampling errors.

The general shape of a curve obtained with the apparatus is often informative. A rapid change in the slope of a curve (upper curve, Fig. 2C) indicates a "critical" point, at which the force applied suddenly shears resistant fibres. It sometimes happens that more than one rapid change of slope shows on the curve, indicating more than one type of fibre. This is illustrated by the upper curve in Fig. 2D; it is interesting to note that although the samples used to obtain both the upper and lower curve were taken from the same muscle, only the sample corresponding to the upper curve showed heterogeneity of fibre type. The relative positions of the rapid changes in slope indicate the relative toughness of the different types of fibre.

Experimental Results

Samples of raw leg of pork and loin beef were adjusted to different pH values by injections of lactic acid or ammonia solutions of appropriate concentrations (7). Each sample weighed about 200 gm. and was given 8 to 10 injections of 1 ml., each distributed as evenly as possible throughout its mass. The samples were stored at 0° C. for four days, after which two sub-samples

were cut from each for tenderness measurements. Hydrogen ion concentration was measured on the sub-samples with a glass electrode.

The results of three experiments with pork and beef, each experiment being made with meat from different animals, are shown graphically in Fig. 3. Each point on a curve represents the mean of two determinations, made on different sub-samples from the same sample; the standard deviation of the mean is ± 0.15 for loin beef and ± 0.23 for pork, calculated from 21 and 30 pairs of duplicates having mean values varying from 1.0 to 3.4 and 1.3 to 3.6, respectively.
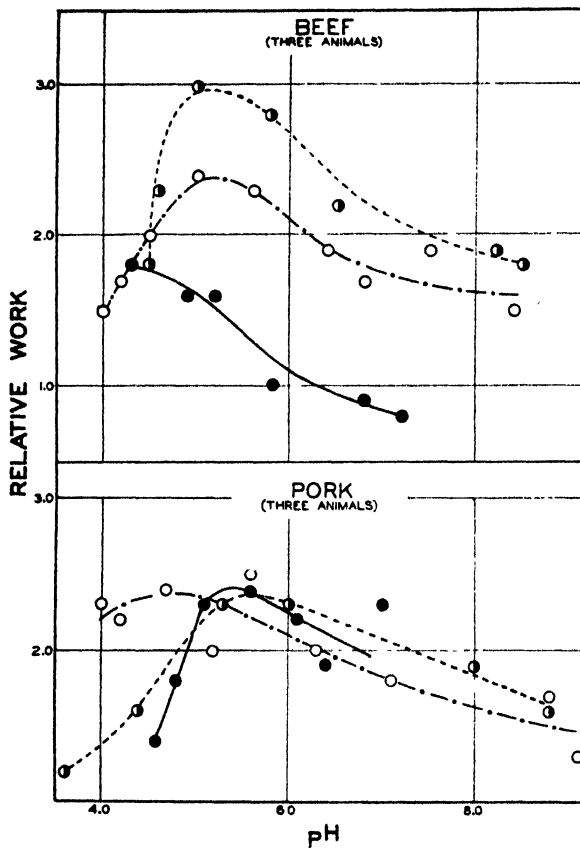
FIG. 3. *Relation between pH and tenderness for pork and beef.*

Discussion

The sampling error in these experiments is large in comparison with the precision of the method used for estimating tenderness. With the technique employed, there were three factors that might have contributed significantly to the sampling error, and should, therefore, be considered: (*i*) variability in initial tenderness of the samples from which the sub-samples for pH and tenderness measurements were taken; (*ii*) non-uniformity of pH throughout

the sub-sample; (*iii*) the presence of striations of connective tissue in the sub-samples.

The influence of the initial tenderness on the final tenderness after adjusting the pH was not studied. However, it will be noticed in Fig. 3 that for a single experiment the deviation of any experimental point from the curve is not greater than the standard error for duplicate determinations at a given pH, *i.e.*, for duplicate determinations on a single injected sample of meat. As there is no reason to suppose that the initial tenderness varied more within a sample than between samples, the main error is probably in taking the sub-samples, including the non-uniformity of pH. The variability in initial tenderness would therefore seem to have little or no effect on the results obtained after injection. This is not improbable, because the variations in initial tenderness of samples from a given muscle are not likely to be large.

Non-uniformity of pH is accounted for by the slow rate of diffusion of lactic acid or ammonia through the meat. It would not be reliable to estimate tenderness at the identical position at which pH was measured in the sub-sample, as insertion of the electrodes undoubtedly ruptures some of the tissue. An average value for the pH was therefore obtained from at least two determinations made beside the line along which the sample was cut. It is estimated that this average pH might differ by $\pm$ 0.03 pH from that along the line of cut.

The error that striations of connective tissue would introduce into an estimate of tenderness can usually be avoided with reasonable care in selecting the sub-sample. Occasionally, connective tissue though present is not visible until the sample is cut, and a spurious result is obtained. Two pairs of duplicate determinations were rejected on this account from 55 pairs made during the present investigation.

In general, the curves in Fig. 3 show that the addition of sufficient lactic acid or ammonia to raw pork or beef made the meat more tender. The failure of one curve for beef to show a maximum might be due to experimental error, or possibly the maximum toughness, *i.e.*, minimum tenderness, for the beef used was not attained. The results suggest that for samples at the same pH from different animals, there is a greater variability in tenderness of beef than of pork; the variability observed with beef is, in fact, greater than the change in tenderness brought about by changing the pH within the limits of about 5.5–6.5 generally observed in beef carcasses. There is also some indication that the pH of maximum toughness is lower for beef than pork. More work with both meats would be necessary, however, to determine whether the indicated differences in variability between animals and pH of maximum toughness are significant.

It is not possible to account for the results depicted in Fig. 3 without additional data to supplement the pH—tenderness measurements. It seems unlikely, from the nature of the curves, that hydrolysis of connective tissue around the fibre bundles is responsible, to more than a minor extent, for the observed changes in tenderness. The changes might be associated with

changes in protein-water relations or possibly with increased activity of protein-splitting enzymes.

Acknowledgment

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COLOUR OF MEAT: II. EFFECT OF DESICCATION ON THE COLOUR OF CURED PORK[1]

BY C. A. WINKLER[2]

Abstract

In the absence of air, a linear relation, independent of temperature, was observed between moisture loss and colour change. The change was mainly one of intensity, and was reversible. In air, irrespective of its humidity, changes in both intensity and quality of colour occurred, but no definite relation was found between changes in humidity and colour quality for different samples. In saturated air, intensity changes became complete in the early part of the storage period, but the enhanced changes in air of lower humidity continued to increase. No influence of temperature on the rate of colour change was observed in dry air or in air of 60% relative humidity.

Introduction

The two more important factors contributing to the surface discolouration of meat are methaemoglobin formation and desiccation. The formation of methaemoglobin on the surface of bacon has received considerable attention, notably by Brooks (1), but the effect of desiccation on the colour of this meat seems not to have been quantitatively investigated. Some results that were obtained during a study of this problem are given in the present paper.

Apparatus

Colours were measured with the photoelectric colour comparator previously described (4). With this instrument the amount of light scattered at right angles to the surface of the sample in the blue, green, and red portions of the spectrum is measured and expressed as a percentage of the amount similarly scattered in the same spectral regions from a standard white surface under the same light intensity. The spectral regions are defined by standard colour filters in the path of the scattered light. The filters used in the present work transmitted the following wave lengths: Blue, 4000–4500Å; Green, 4900–5800Å; Red, 5750–7000Å.

Experimental Methods and Results

Colour Change by Vacuum Drying

Moisture is lost at a greater rate from the surface than from the interior of a slice of bacon when drying occurs. Therefore, to make a quantitative

study of the relation between change in surface colour and amount of desiccation of sliced bacon, it is necessary either to assume that the moisture lost from the surface layer is directly related to the total moisture loss, or to use very thin slices.   There is no reason to suppose the first alternative to be a valid assumption, and the second alternative is difficult to adopt experimentally.   By the use of minced bacon, however, the average colour change corresponding to a given loss of moisture throughout the sample can be obtained, and minced bacon has therefore been used in several of the experiments to be described.

Weighed samples (about 50 gm. each) taken from the same batch of minced, unsmoked bacon were dried in *vacuo* at 0° C., 10° C., and 25° C.   Each sample was periodically weighed, thoroughly mixed and its colour measured.   A plot of the results for a typical experiment is shown in Fig. 1, from which it is evident that the relation between colour change and moisture loss is linear and independent of temperature. The change in colour is mainly one of intensity rather than quality; this is inferred from the fact that a decrease of about 43% in scatter of blue, green or red, corresponding to a 50% moisture loss, is accompanied by not more than 6% change in the ratios of scatters, *i.e.*, red/green, red/blue, or green/blue.

The colour change caused by vacuum drying was found to be reversible when partially dried samples of sliced or minced bacon were placed in an atmosphere of water vapour at 10° C. or 25° C.   Typical results are shown in Table I.

FIG. 1.   *Relation between colour change and moisture loss* in vacuo.

TABLE I
REVERSIBILITY OF COLOUR CHANGE AFTER DRYING *in vacuo*

Condition	Time of exposure, hr.	Red scatter, %		Green scatter, %		Blue scatter, %	
		10° C.	25° C.	10° C.	25° C.	10° C.	25° C.
Initial colour	–	34.0	33.4	24.2	24.2	22.0	21.2
Moisture loss	2	–	29.8	–	21.5	–	18.0
Moisture loss	12	26.0	27.8	19.0	18.9	18.6	17.8
Moisture gain	12	28.4	29.1	20.0	20.8	19.4	18.6
Moisture gain	24	31.2	–	21.3	–	20.4	–
Moisture loss	16	27.3	–	18.2	–	17.0	–
Moisture gain	54	–	31.0	–	22.5	–	20.2
Moisture gain	96	34.0	–	24.4	–	22.4	–

At 10° C. the reversibility was complete, providing the samples remained in contact with the water vapour a sufficient length of time. At 25° C., formation of bacterial slime on the surface of the meat always preceded complete recovery of the initial colour, otherwise the behaviour was similar to that observed at the lower temperature.

Colour Change in Air at Different Relative Humidities

In the vacuum drying experiments, the absence of oxygen precluded colour changes due to methaemoglobin formation. If, however, bacon is stored in air at 100% relative humidity, the formation of methaemoglobin should be the only factor responsible for colour change. On the other hand, the changes observed in air at lower relative humidities should represent the combined effects of drying and methaemoglobin formation.

Several experiments were made to determine the nature and relative amounts of the colour changes resulting from oxidation alone, and from oxidation and drying combined. For each experiment, duplicate samples of about 50 gm. each, taken from the same batch of minced, unsmoked bacon, were stored at room temperature in desiccators through which a slow stream of either saturated or dry air was passed. At intervals, each sample was thoroughly mixed and the amount of colour change determined. The results of a typical experiment are plotted in Fig. 2A.
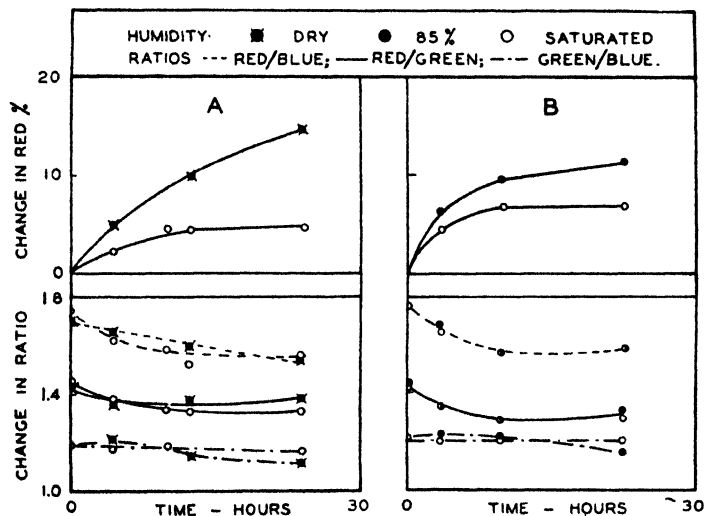
Fig. 2. *Effect of storage in dry and moist air on colour intensity and quality.*

Unlike storage *in vacuo*, storage in dry or saturated air produced marked changes in quality (upper curves) as well as in intensity of colour (lower curves). In air of 100% relative humidity, change in colour intensity, as inferred from change in red scatter, always attained a maximum value, presumably after methaemoglobin formation had gone to completion. In

dry air, the colour intensity continued to change throughout the storage periods used.

The quality of the colour developed in dry air usually differed somewhat from that developed in saturated air, for samples taken from the same batch of minced bacon. However, the results of a number of experiments with samples from different batches failed to indicate any definite relation between the quality of the colour and relative humidity. This is perhaps to be expected, as the rate of methaemoglobin formation probably depends upon both the rate and extent of drying (2), and these two factors will almost certainly vary from sample to sample.

While the experiments in dry and saturated air were satisfactory for determining the nature and relative magnitudes of the changes in colour of bacon stored under extreme humidity conditions, it was thought desirable to ascertain also the extent to which drying influenced the colour when the humidity approached that used in commercial practice. Experiments were therefore made in which samples of minced, unsmoked bacon were stored at room temperature in slow streams of air conditioned to 85% and 100% relative humidity. The results for a typical experiment are plotted in Fig. 2B. It is evident from the curves for both change in ratio and change in red scatter that during the first few hours' storage at 85% relative humidity, methaemoglobin formation is responsible for most of the observed colour change. Subsequently, however, the effect of drying on colour intensity becomes important, as shown by the increased change in red scatter. With a given sample of bacon, there is little difference in the quality of the colour developed during storage at the two humidity levels.

A few experiments were also made with slices of unsmoked and smoked bacon in air of 60% and 100% relative humidity at 10° C. The results obtained with both these meats in the sliced condition were similar to those shown in Fig. 2B, except that the effect of drying was more marked at the lower humidity and lower temperature. Changes in colour quality were again found to be similar at the two humidity levels.

Effect of Temperature

The effect of temperature on the rate of colour change under drying conditions was studied by storing 50-gm. samples of bacon, both smoked and unsmoked, in a slow stream of dry air at 10° C. and 25° C. Results for typical experiments are shown in Fig. 3. It is apparent that for a given moisture loss from each type of meat, the change in colour intensity is the same at both temperatures. Although slight differences in quality were observed, there was no apparent relation between changes in temperature and colour quality with different samples. Experiments were also made with slices of bacon stored in air of 60% relative humidity at 0° C. and 10° C. Again, no influence of temperature on the rate of colour change could be observed in the temperature interval used.

It is somewhat surprising that the part contributed to the colour change by methaemoglobin formation is not sufficiently different at the different temperatures to be revealed. Presumably the effects due to drying masked the discolouration resulting from methaemoglobin formation, since the changes observed in colour quality indicate that methaemoglobin was formed. It might well be that drying in air of higher humidity would permit a temperature coefficient of colour change to be detected. It might be mentioned,
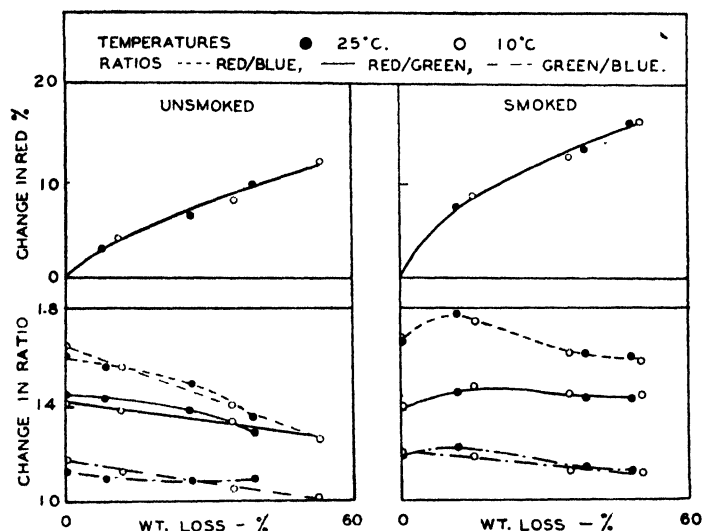
FIG. 3. *Effect of temperature on colour intensity and quality under drying conditions.*

however, that some preliminary studies of the influence of temperature on methaemoglobin formation in minced bacon stored in saturated air gave very variable results. With some samples, temperature appeared to have no influence, while with others the rate of methaemoglobin formation increased two to three times for an increase of 10° C.

Discussion

Quantitatively the results of this investigation are applicable only to small samples, but certain qualitative generalizations are also possible. When the results are considered as a whole it is evident that in the absence of air and under conditions that prevent desiccation, the colour of bacon should be subject to change only through putrefaction, a conclusion essentially similar to that arrived at by Brooks (1) from other experimental evidence. In the presence of either dry or moist air, however, methaemoglobin formation causes changes in both intensity and quality of the surface colour, the intensity changes but not the quality changes being enhanced by drying.

The effect of desiccation becomes increasingly significant as the storage period is prolonged. Methaemoglobin formation, however, seems to attain to

a maximum value, although a more sensitive instrument than that used might show a slow formation of methaemoglobin following the rapid initial change. According to Brooks (1), methaemoglobin formation is confined to a superficial layer about 5 mm. in thickness, but there is no reason to suppose that the effect of drying is similarly limited, since loss of moisture undoubtedly occurs from the body of the sample. Loss of considerable moisture from the interior of the meat, *i.e.*, in the absence of oxygen, might result simply in changes in the intensity of the internal colour, but there is some evidence (5) that loss of even a relatively small amount of moisture might have profound effects on the colour of freshly cut surfaces.

The reversibility of the changes in colour intensity caused by vacuum drying indicates that the meat pigments are not appreciably denatured by desiccation at ordinary temperatures, and the suggestion of Heiss and Hohler (3) that cathaemoglobin is formed during the drying process seems rather improbable. It seems unlikely also that these results, obtained with minced bacon, can be satisfactorily explained on the assumption that intensity changes caused by drying are due solely to optical effects (2). Concentration of the pigments by removal of water would seem to afford a more satisfactory explanation of both the observed changes and their reversibility.

Acknowledgment

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DEW-POINT HYGROMETER FOR USE AT LOW TEMPERATURES[1]

By C. A. Winkler[2]

Abstract

An apparatus is described in which provision for slow cooling of a metal mirror by circulating over it liquid from a vessel in a thermoregulated bath, and the use of multiple thermocouple elements contained in the mirror, enable the dew-point temperature to be gradually approached and accurately determined. Precise measurements of relative humidity at low temperatures, where the moisture content of the air is small, are therefore possible. A precision of ±0.5% relative humidity was readily attained at temperatures down to −15° C.

Introduction

Accurate measurement of relative humidity, as an essential aspect of its accurate control, is important in relation to the refrigerated preservation of perishable products. Although many instruments, based on several different principles, have been developed for the measurement of humidity (2), many of these are not sufficiently sensitive to permit precise estimation of the moisture content of air at low temperatures, while others of greater sensitivity are generally empirical and require calibration. There is, therefore, an obvious need for an instrument based on sound theoretical principles and capable of accurate determination of relative humidity at low temperatures. These considerations led to the development of the absorption and dew-point instruments described in the present paper.

Description of Instruments

Absorption Hygrometer

Attempts were made to develop an absorption hygrometer suitable for determining the humidity of air samples drawn from packaged products stored at low temperatures. The apparatus was designed to prevent both access of the sample of air to the desiccant during sampling, and volume changes in the system when the desiccant was brought into contact with the sample. In its final form, the hygrometer was entirely satisfactory in technical details and for use at ordinary temperatures, but the results obtained with it at temperatures below 0° C. were erratic. After a number of unsuccessful attempts to overcome the eccentricities, it was finally concluded that these were due to variable adsorption of moisture on the walls of the test chamber during sampling. Further development of the instrument must therefore be postponed until this difficulty can be overcome.

Dew-point Hygrometer

The two main difficulties encountered in the application of the dew-point principle to the determination of relative humidity at low temperatures are:

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(i) The observed temperature must yield an accurate estimate of the true temperature of the surface on which dew is deposited, since the dew-point depression per unit change of humidity decreases with the air temperature. (ii) The small amount of moisture present in the air at low temperatures increases the time required for a visible deposit of dew to form, and unless the dew-point temperature is approached very gradually its observed value may be considerably below the true value.

Awbery and Griffiths (1) have described a dew-point instrument in which the first of these difficulties is largely overcome by pressing a thermocouple junction against the mirror on the reverse side to that on which dew is de-deposited. The rate of cooling of the mirror was only partially controlled, however, by regulating the rate of flow of paraffin oil, cooled with solid carbon dioxide, across the reverse side of the mirror. These authors observed a mean difference of 3% in relative humidity between the results obtained with the dew-point instrument and those obtained by the standard gravimetric absorption method.

In the instrument described in the present paper the difficulties mentioned above are both overcome, the first by having multiple thermocouple junctions contained in the mirror and connected in series to increase the thermoelectric potential developed, and the second by providing accurate temperature regulation of the cooling fluid so that the mirror may be cooled as slowly as desired. The accuracy attainable with the apparatus in its present form depends upon the rate at which the dew-point temperature is approached.

The principle of the apparatus can best be understood by reference to Fig. 1. The vessel C is filled to the indicated level with a non-freezing solution such as alcohol or ethylene glycol in water. The rest of the assembly, consisting of the liquid-circulating system, dew-point mirror, and thermocouples, is then put into place. In order to circulate the liquid, air is forced through the inlet tube A and passes out of the jet J below the liquid level. Air bubbles and liquid entrapped between them pass up through the tube B, across the under side of the mirror M to cool it, and down through the tube D, the liquid returning to the vessel C while the air escapes at E.

The mirror is made of copper, thinly plated with chromium, and has six holes drilled into its edge, each hole to accommodate a thermocouple junction T of 40-gauge copper and constantan wires. When in position, the junctions lie about 1 mm. beneath the surface of the mirror, and are held fast and insulated from the apparatus by a thin film of shellac. The mirror can be conveniently mounted by drilling two longitudinal holes in a brass rod, and recessing a portion of this rod as shown in the diagram. It has been found most convenient to immerse the second set of thermocouple junctions in a non-freezing solution in a Dewar vessel, and obtain their temperature with an accurate thermometer. The two sets of junctions are connected in series with a galvanometer calibrated to read directly the temperature difference between them.

The assembly shown in the diagram is immersed slightly beyond the liquid level in C in a non-freezing liquid bath provided with a small electric heater, and surrounded by a cooling medium. In practice, an ice formed by freezing a 23% solution of sodium chloride (F.P. $-21°$ C.) has been found satisfactory for air temperatures down to $-15°$ C. Solid carbon dioxide may also be used. With either of these substances a small air space separating the liquid bath from the cooling medium to retard the rate of cooling is an advantage. Cooling by mechanical means has not been attempted but should also be satisfactory. Accurate control of the cooling rate is obtained by manual control of the electric heater in the bath.
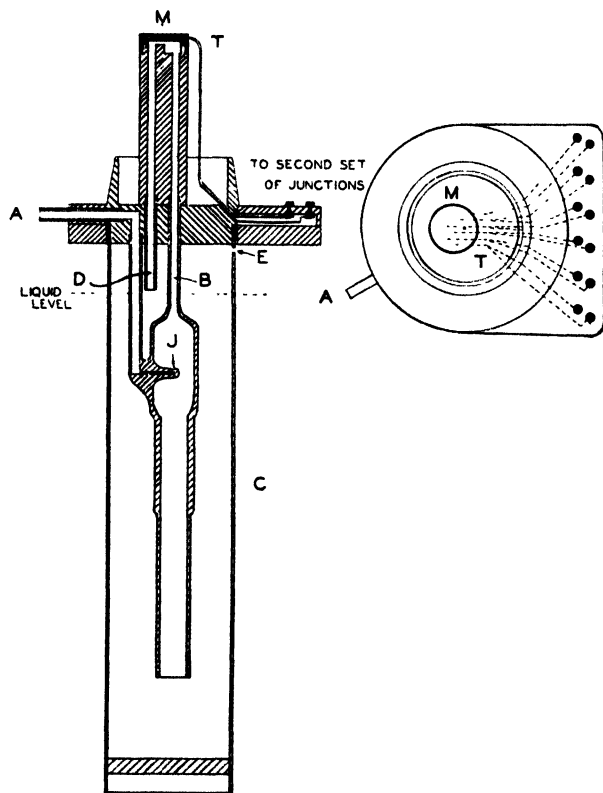
FIG. 1. Diagram of dew-point hygrometer.

An air stream of about two bubbles per second from a jet 0.045 in. in diameter is sufficient for satisfactory circulation of the liquid through the mirror. A small compressor with a safety valve and stopcock in the line to provide control has been found convenient, but a variety of other methods could doubtless be used to obtain the necessary slow stream of air. Displacement of air from a bottle by a liquid under a small head pressure, compressed air, or even a mechanically operated syringe bulb should serve the purpose.

Tests with a manually controlled arrangement of the apparatus at temperatures down to −15° C., and over a range of 60 to 95% relative humidity showed that a precision of ±0.5% relative humidity was readily attained when the dew-point was approached at the rate of about 2° C. per hour. Although precision of this order is quite adequate for most practical purposes, it could doubtless be further increased by approaching the dew-point more slowly. There is no reason to believe that the apparatus would not work equally well at considerably lower temperatures. By providing photo-electric detection of dew formation it should be possible to incorporate the principle into an automatically operating and recording instrument. The development of such an instrument has been undertaken.

Acknowledgments

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A STUDY OF BACTERIA CONTAMINATING SIDES FOR WILTSHIRE BACON WITH SPECIAL CONSIDERATION OF THEIR BEHAVIOUR IN CONCENTRATED SALT SOLUTIONS[1]

BY E. H. GARRARD[2] AND A. G. LOCHHEAD[3]

Abstract

Forty microbial types, in which micrococci predominated, were found as representative of pre-curing contamination, including strains resembling organisms found in bacon slime.

Varying degrees of salt tolerance were noted, micrococci showing the greatest ability to grow at higher concentrations. With 25% sodium chloride only two species showed growth. Tests with species reducing nitrate to nitrite at 5% salt concentration showed that as the salt concentration increases, nitrate reduction occurs with a progressively smaller proportion of those showing growth. This suggests that nitrate reduction in curing pickle is a function of the true halophiles rather than of the pre-curing contaminants.

The organisms could be placed in five groups depending upon their salt resistance (ability to survive) in salt solutions and in curing pickle. Much greater resistance to salt was displayed in curing pickle than in salt broths of similar sodium chloride content. Pickle appeared to possess substances tending to neutralize the toxic effect of salt, the action being "protective" rather than "stimulative".

Many types of bacteria constituting original contamination are considered able to survive the pickling process. Although the findings do not point to any pronounced activity of these in pickle, their ability to survive opens the possibility of their becoming active after pickling and contributing to storage defects. The results justify the adoption of measures for the utmost plant sanitation in Wiltshire processing.

Introduction

The greater part of the bacon exported from Canada is in the form of Wiltshire sides, which are pickled, drained, and baled in Canada and receive further treatment in England. From time to time, sides when received are found to show off-flavour, discoloured areas, or slime, the latter principally on the membrane of the rib tissue. Bacteriological examination of slime may show considerable numbers of micro-organisms, many of which are non-halophilic in nature, despite the fact that the sides have been cured in strong brine and, after draining, have a salt content of some 4 to 5%. This suggests that undesirable bacteria either may be present in the pickle or may contaminate the sides during draining, wiping, and baling operations. The extent of the defects on the sides will depend naturally on their development as affected by temperature, humidity, and time of storage before arrival of the sides on the market.

It is well known that carcasses may be contaminated during operations on the killing floor of slaughterhouses and packing plants and that this contamination may be augmented by subsequent handling of the meat. It has

been shown by Haines (2) that the initial bacterial load noticeably affects the storage life of the meat. Obviously the storage life will also be dependent upon the extent to which contaminating bacteria have been allowed to develop. The importance of such factors as temperature, relative humidity, and carbon dioxide concentration during storage has been shown by a number of workers, such as Schwartz and Schmid (5), Haines (1), and Scott (6, 7, 8). For the fresh and chilled meat trade, the prevention and control of microbial contamination are thus matters of the greatest concern, and standards of cleanliness are advised to keep contamination by, and growth of, micro-organisms at a minimum.

In Wiltshire processing, involving the curing of sides in concentrated brine under conditions in which bacteria are considered essential agents, the significance of the initial contamination is by no means clear. In our packing plants, hygienic measures are adopted to minimize contamination prior to curing, on the justifiable assumption that micro-organisms contaminating meat, if allowed time and suitable temperature, may cause various types of spoilage. However, there is little or no exact knowledge of the bactericidal or bacteriostatic effect of the high salt content of the curing pickle on contaminating organisms, nor on the more practical questions as to the possible role of such contaminants in the cure, or the ability of certain types to survive the pickling and act as possible agents of spoilage during the subsequent storage period.

Preliminary studies (4) in the analysis of curing pickle have shown the presence in the brine of appreciable numbers of non-halophilic organisms, able to resist, if not multiply in, high concentrations of salt. Observations (unpublished) on the surface load of Wiltshire sides at different stages of the processing showed that organisms able to develop without salt were an important group of bacteria prior to pickling, and likewise comprised a group that definitely increased during the storage of the pickled sides after baling. Taken together, the above sets of findings suggest that deleterious organisms that may contaminate sides prior to pickling, may carry through and cause defects on cured, stored sides. The aim of the present study was to obtain information of a more direct nature on this point.

Experimental

The main purpose of the experiments here reported was to study the types of bacteria contaminating sides of Wiltshire bacon prior to curing, and to note particularly their ability to tolerate various concentrations of salt, with distinction between capacity for growth and for survival. Samples were taken from sides just prior to pumping and pickling, as being representative of plant contamination preceding the cure. To afford further information and obtain representative types of organisms from various sources of contamination, samples were also taken from freshly slaughtered hogs, from sides in the chill room just before removal to the cutting room (approx. 40 to 48 hr. after killing), and from sawdust, air, and wall scrapings in the chill room.

Surface contamination of the sides was studied by a filter paper impression method, using Whatman No. 3 filter paper cut into squares of 4 sq. cm. and sterilized in Petri dishes in hot air at 120° C. Samples were taken by pressing a square of paper firmly against the meat tissue for 20 sec. with sterile forceps, and then dropping it into a 750-ml. Erlenmeyer flask containing 500 ml. physiological salt solution and 75 gm. broken glass. Various methods of estimating surface contamination of meat have been used. The impression method was selected in preference to those involving scraping, or cutting out measured areas, as it obviated any mutilation of the tissue. From each side, impressions were taken as follows: two from lean flesh, two from centre rib section and one from lower short ribs. To each flask were added 20 squares representing impressions from four sides. Hogs were grouped into lots of four, picked at random as they came from the killing and dressing floor on their way to the cool room. From each group (eight sides) two flasks were prepared. In all, 32 sides were used for sampling, marked so they could be followed to the cutting room.

At the laboratory, flasks were shaken for 10 min. until the filter paper was disintegrated, and the contents of each pair of flasks were mixed in a previously sterilized 2000-ml. flask. From the combined suspension of 40 squares, further dilutions were prepared with physiological saline blanks, and plates were poured using nutrient agar and agar media containing respectively 5, 10, and 15% sodium chloride*. All plates were incubated at room temperature (approx. 20° C.). Nutrient agar plates were counted after five days, 5% and 10% salt plates after two weeks, and 15% salt plates after three weeks. Similar media were used for the quantitative determination of organisms in sawdust, air, and wall scrapings of definite areas.

Pre-curing Contamination

Though the main object of the work was to obtain representative types of bacteria for detailed study rather than a systematic quantitative examination of plant contamination, quantitative data illustrating the trend of surface contamination at different times are shown in Table I. In Table II is found a summary of results from the examination of sawdust, wall scrapings, and air.

As expected from an examination of this kind, in which the extent of contamination is varied and accidental direct contact with walls, clothing, etc., may make for great localized differences, variations are noted. The method of sampling involves additional errors due to the possibility of touching the same area twice and of covering more grossly contaminated spots from which contamination may be later reduced in various trimming, cutting, and wiping processes. The results show, however, that sides for Wiltshire bacon may become definitely contaminated with bacteria prior to pickling, within the space of some 40 to 48 hr.

* *In this paper, % sodium chloride = grams per 100 ml. of solution.*

The findings suggest that under conditions in which sides are removed, promptly after killing, to a chill room of approximately 32° F., the microbial load at the end of cutting is the result of direct contamination rather than growth. It is possible for sides to leave the chill room with little or no increased microbial load; when increased counts are noted, they are regarded as due largely

TABLE I

SURFACE CONTAMINATION OF WILTSHIRE SIDES PRIOR TO CURING

Group No.	—	Count per sq. cm. on agar			
		0% NaCl	5% NaCl	10% NaCl	15% NaCl
1	Freshly killed	87	87	19	6
	After cooling	1,110	1,090	450	220
	After cutting	3,890	4,860	2,260	790
2	Freshly killed	3,550	5,350	3,031	750
	After cooling	31,500	40,560	5,150	100
	After cutting	3,750	2,030	1,820	340
3	Freshly killed	630	220	100	50
	After cooling	660	150	50	20
	After cutting	12,130	7,440	1,220	490
4	Freshly killed	150	190	170	40
	After cooling	250	130	70	6
	After cutting	10,810	7,810	2,140	840

to direct contact infection.   Table II suggests that air is normally a relatively insignificant source of contamination, whereas the numbers of bacteria present in used sawdust or in wall scrapings point to the danger of greatly increased contamination of the meat resulting from accidental contact with such sources. The increased counts after cutting are due to the handling in the cutting room involving various cutting, sawing, trimming, and handling operations carried out during a period of approximately one-half hour.

TABLE II

BACTERIAL COUNTS—VARIOUS SOURCES OF CONTAMINATION

Source	Agar plate count			
	0% NaCl	5% NaCl	10% NaCl	15% NaCl
Fresh sawdust, per gram	7,000	9,000	1,000	1,000
Used sawdust, per gram	216,000,000	190,000,000	73,000,000	750,000
Bloody sawdust, per gram	14,400,000,000	24,640,000,000	8,800,000,000	700,000
Wall scrapings, per gram	67,500,000	47,500,000	46,200,000	38,700,000
Exposed plates[1]—floor	180	125	15	5
Exposed plates—raised	90	33	23	15

[1] Area, 60 sq. cm.; exposed to air 15 min.

Classification of Organisms

Examination of the plates of nutrient agar and of salt agar showed variations in the bacterial types developing. Special attention was given the "after cutting" series in an estimation of the relative incidence of different types. From plates or sectors of plates all colonies were picked and transferred to agar slants for further study. The percentage distribution of the various morphological types is illustrated in Fig. 1, which shows the changing proportions of the groups as they vary with the salt content of the medium.
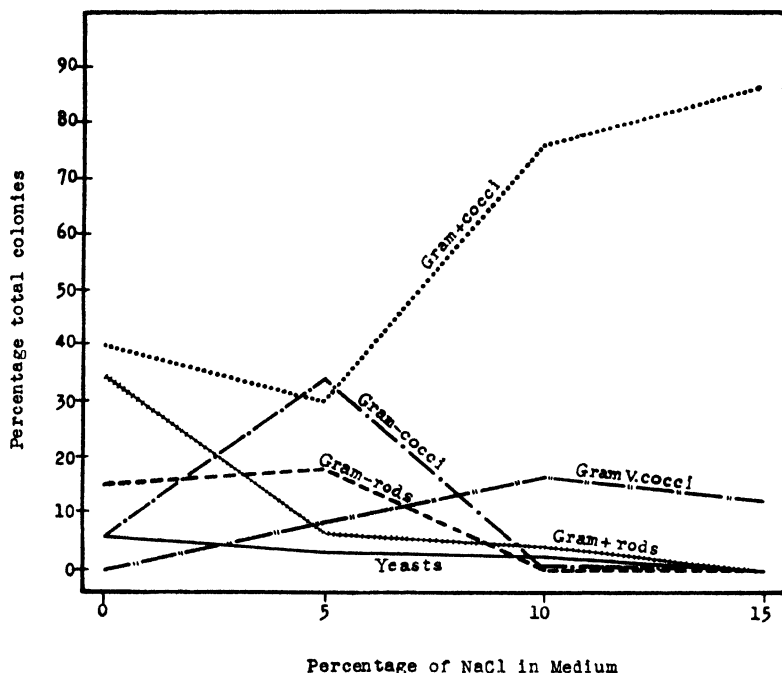
FIG. 1. *Distribution of various morphological types on plates of different salt concentration, representing contamination of sides after cutting.*

For more detailed study of the hundreds of colonies isolated it was necessary to concentrate on one isolation group. It was felt that the organisms on 5% salt agar plates would be most representative of those comprising initial contamination of meat to be pickled. This medium showed a well-assorted bacterial flora, with all morphological groups well represented, and gave comparatively high total counts. Facultative halophiles, which might be important in pickle, should be well represented on 5% salt agar, to the exclusion of certain non-halophiles. Finally, as 5% salt is a favourable concentration for isolating types occurring in bacon slime, it was felt that this concentration would be most likely to include organisms able to cause spoilage. Accordingly, 100 cultures from the 5% salt agar plates of the "after cutting" series, together with a group of 185 cultures from plates of the same medium prepared from sawdust, air, wall scrapings, and freshly slaughtered hogs,

were studied microscopically and culturally with the object of eliminating similar strains and reducing the number to those that could be regarded as different type species. For this differentiation, reliance was placed on Gram staining, nitrate reduction, gelatin liquefaction, lipolysis, litmus milk reaction, and fermentation of dextrose. All test media contained 5% sodium chloride.

The relative abundance of the morphological groups represented in the 285 cultures isolated from 5% salt agar plates is shown in Fig. 2, organisms from the "after cutting" series being compared with those from various sources before cutting. Micrococci, Gram positive and Gram negative, were found to comprise the most abundant groups, followed by Gram negative rods,
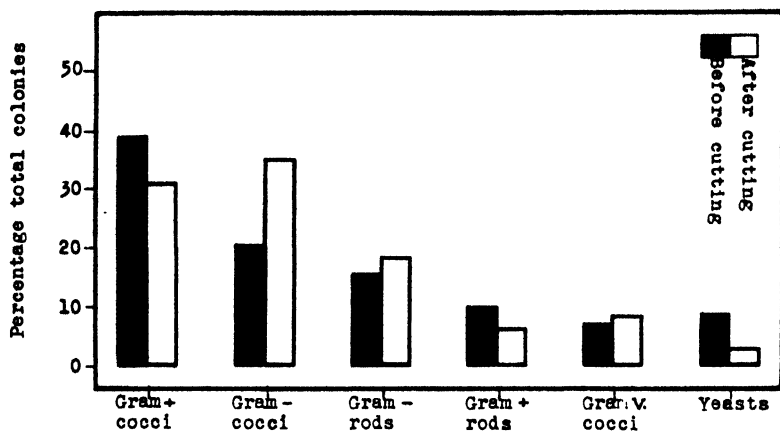
FIG. 2. *Relative incidence of different groups of organisms from various sources before cutting, and from the "after cutting" series.*

many of which were highly pleomorphic forms. A numerically important group, the classification of which presented some difficulty, was included as Gram negative cocci. These organisms were characteristic oval forms which might also be regarded as coccoid rods. As a group these showed relatively pronounced lipolytic action and were similar to organisms found by Landerkin (3) to be the most abundant types appearing on 5% salt agar plates from certain samples of slime on cured Wiltshire sides.

As a result of the comparative study of 100 cultures from the "after cutting" series, 28 bacterial cultures and one yeast were regarded as different types. Examination of the group of 185 cultures revealed 11 additional bacterial types. Forty cultures therefore were regarded as types representative of the 285 original isolations. Further study was made of the cultural characteristics of these organisms, details of which need not be given here. However, a summary of the 39 bacterial types representing pre-curing contamination is given in Table III, the organisms being grouped on the basis of morphology and more important physiological properties.

TABLE III

SUMMARY OF BACTERIAL TYPES REPRESENTING CONTAMINATION PRIOR TO CURE

—	No. of different types	Chromogenic	Motile	Spores	Gelatin liquefaction	Nitrate reduction	Lipolysis	H₂S production	Acid in		
									Dextrose	Sucrose	Lactose
Micrococci, Gram pos.	10	5	0	0	5	6	6	4	8	7	4
Micrococci, Gram neg.	6	0	0	0	0	4	5	2	0	0	0
Micrococci, Gram. var.	4	0	0	0	1	2	1	1	4	4	2
Rods, Gram. pos.	9	2	0	1	4	4	1	1	2	2	0
Rods, Gram neg.	10	2	4	0	3	6	2	3	3	2	0
Total	39	9	4	1	13	22	15	11	17	15	6

Relation of Organisms to Salt

The effect of salt on micro-organisms has been given much attention and there has accumulated, in consequence, a voluminous literature which need not be reviewed here. It is known that organisms from a salt-free environment can tolerate salt to varying degrees, with evidence of some stimulation by lower, and eventual suppression by higher concentrations. The degree of tolerance to salt is further modified by other environmental factors such as temperature, aeration, pH, organic nutrients, amount of inoculum, age of culture, and microbial association. It is further apparent that certain types, such as micrococci, from salt-free sources are able to withstand higher salt concentrations than others. From salt environment, on the other hand, both from natural and "industrial" sources, organisms have been found that prefer or require various concentrations of salt, some thriving best in an environment approaching saturation. Among such organisms micrococci are less predominant. That a considerable degree of adjustment to salt may occur seems well established, though there is still difference of opinion as to the suitability of, and distinction between, such terms as "obligate halophile", "facultative halophile", "salt tolerant", and "salt resistant".

Since organisms contaminating Wiltshire sides prior to curing are subjected to the influence of a salt pickle approximating saturation, it was considered a matter of practical interest to study the relation of the microbial types found, in various concentrations of sodium chloride. Information on this point should permit a better estimate of the importance of pre-curing contamination and its possible relation to later defects of the cured product. Tests were therefore made of the "salt tolerance" and "salt resistance"* of 40 microbial types representing contamination, to which were added, for comparison, seven other organisms from various sources.

* For the purpose of this paper, "salt tolerance" refers to ability to grow, and "salt resistance" ability to remain viable, in an environment of definite salt concentration.

METHODS

Salt tolerance. From eight-day cultures of the organisms on 5% salt agar, faintly turbid suspensions were made in 5% sodium chloride solution, and one loopful of the suspension was used for inoculation. Heavy inocula were avoided, in view of the findings of certain workers (9, 10), who showed that mass effect caused by the addition of large inocula may increase salt tolerance, and thus tend to neutralize any inhibitive effects. Transfers were made to a series of slants of nutrient agar containing respectively 0, 5, 10, 15, 20, and 25% sodium chloride, in addition to 0.1% potassium nitrate. Transfers were also made by loop stab to a similar series of semi-solid media of the same composition except that 0.08% agar was used. Although the amount of agar used was so small that the medium did not appear to differ from straight broth, tests showed that when growth occurred it appeared as a suspended localized growth, which showed to advantage compared to a general distribution in broth, and made the recognition of bacterial multiplication easier.

Inoculated tubes were incubated at room temperature for two weeks in the case of 0 and 5% sodium chloride media, and for three weeks with the higher concentrations. Tightly rolled plugs allowed but a minimum of evaporation. The tubes were examined for growth and tested for nitrites. Organisms showing growth were adjudged to have tolerated the salt concentration in question.

Salt resistance. By means of a measured loop, equivalent amounts of 10-day cultures were transferred to 10 ml. of 5% sodium chloride solution. This was agitated and an endeavour made to establish as uniform turbidity as possible. One-tenth ml. of suspension of each culture was transferred to two tubes of 5% sodium chloride broth, to one tube each of 10, 20, and 30% sodium chloride broth and to one tube of sterile pickle (29.75% sodium chloride). The salt broths contained, in addition to sodium chloride, 0.1% potassium nitrate, 0.15% beef extract, and 0.25% peptone. The pickle was a filtered curing brine, as used for Wiltshire sides. All tubes contained 10-ml. quantities.

From one of the duplicate 5% sodium chloride tubes, plates were poured with 5% salt agar and incubated at room temperature for two weeks. The counts gave the original count per ml. at time of inoculation. At the end of 5 and 10 days' incubation at room temperature, times that correspond approximately with the duration of cure at various plants, estimates were made of the numbers of organisms in each inoculated tube by plating on 5% salt agar. Since larger amounts taken from the tubes of the higher salt concentration would affect the sodium chloride concentration and hence the counts of the agar plates, transfers were made by loop. Calibration for each sodium chloride broth was necessary owing to the effect of specific gravity on the volumes delivered by the same loop. Preliminary tests were made in weighing loopfuls of each salt concentration. Repeated weighings showed that out of 120 loopfuls there was rarely more than one or two loopfuls difference each

time.   In plating, the loopful was mixed with sterile 5% salt solution in the plate and gently tapped to rid the loop of all its contents.   This followed analogous procedure in standardizing.   Dilution, if necessary before plating, was made in sodium chloride solution.   Plates were incubated at room temperature for two weeks before counting.

RESULTS

Results from the salt tolerance tests showed good agreement between the agar and semi-solid inoculations.   The semi-solid medium showed if anything slightly better growth, and in Table IV are summarized the results from this medium, arranged for the morphological groups.   Gram negative micrococci show a pronounced decline in salt tolerance between sodium chloride concentrations of 10 and 15%, whereas Gram positive micrococci display a greater salt tolerance.   There was little difference between the Gram positive and Gram negative rods, considered as groups.   The tests show that many organisms isolated on 5% salt agar from an apparently salt-free environment

TABLE IV

SALT TOLERANCE (CAPACITY FOR GROWTH) OF MICRO-ORGANISMS REPRESENTING
CONTAMINATION PRIOR TO PICKLING

Morphological group	No. of types in group	Number showing growth in semi-solid medium					
		0% NaCl	5% NaCl	10% NaCl	15% NaCl	20% NaCl	25% NaCl
Contaminants from plant							
Micrococci, Gram pos.	10	10	10	10	9	3	1
Micrococci, Gram neg.	6	6	6	6	1	1	0
Micrococci, Gram. var.	4	4	4	3	3	0	0
Rods, Gram pos.	9	9	9	9	5	2	1
Rods, Gram neg.	10	9	10	9	5	2	0
Yeasts	1	1	1	1	0	0	0
Total	40	39	40	38	23	8	2
Control Cultures							
Staph. aureus		+	+	+	+	−	−
Esch. coli		+	+	−	−	−	−
Achromobacter sp. from slime (No. 44)		+	+	+	+	+	−
Micrococcus sp. from pickle (No. 42)		−	+	+	+	+	−
Achromobacter sp. from pickle (No. 45)		−	−	+	+	+	+
Micrococcus sp. (No. 46)		+	+	+	+	+	−
Micrococcus sp. (No. 47)		+	+	+	+	+	−

in a packing plant can tolerate relatively high sodium chloride concentrations, more than one-half being able to grow on media ranging from 0 to 15% sodium chloride, and one-fifth up to 20%.   A concentration of 25% sodium chloride, however, appears to be definitely inhibitive to organisms isolated and carried on a 5% sodium chloride medium.   That some adjustment to high salt environment of pickle is possible, however, is suggested by the range of adaptability shown by many types.

Since part of the value of a pickle flora depends upon the presence of nitrate-reducing bacteria, cultures were tested for nitrite. Of those shown in Table IV, 26 species reduced nitrate to nitrite in 5% sodium chloride medium. In addition to the original series with light inoculum, a special series was prepared in which heavy inoculations were made from the 26 nitrate-reducing organisms. Results are shown in Table V. It is of interest to note from the

TABLE V

COMPARISON OF GROWTH AND NITRATE REDUCTION AT DIFFERENT SALT CONCENTRATIONS

26 species reducing NO₂ at 5% NaCl concentration	NaCl concentration					
	0%	5%	10%	15%	20%	25%
Light inoculum						
Growth	24	26	26	15	8	3
Nitrate reduction	24	26	24	11	4	1
Heavy inoculum[1]						
Nitrate reduction	24	26	24	12	6	1

[1] *Owing to heavy inoculum, growth could not be determined in this series.*

"light inoculum" series, that growth up to a concentration of 5% sodium chloride was accompanied by nitrate reduction. As the sodium chloride concentration of the medium increased, however, nitrate reduction occurred with a progressively smaller proportion of the species showing growth, until at 25% sodium chloride concentration the only organism showing reduction was one of the "controls", a halophile isolated from curing pickle. Mass inoculation produced relatively little effect on nitrate reduction. The findings are of interest in showing that nitrate reduction is interfered with before growth is suppressed, and suggest that reduction of nitrate in curing pickle is a function to be ascribed to the true halophiles rather than to the pre-curing contaminants.

In the salt resistance experiments, in which 47 cultures were placed in five salt environments and initial, 5-day, and 10-day (and in one set, 30-day) counts recorded, 564 analyses were made. In portraying this mass of data, ranging from very high to very low counts, it was felt that the findings could best be summarized in, (a) a graph showing the trend of groups according to their behaviour in salt, and (b) a table expressing the reaction according to morphological types. These are shown respectively in Fig. 3 and Table VI.

According to the action of the organisms in salt solution, five distinct trends were shown by the counts (Fig. 3).

Groups 1 and 2. Closely related groups, comprising nine species of Gram positive micrococci, four Gram positive and two Gram negative rods, which maintained their numbers relatively well in 20% and 30% sodium chloride and in pickle. Group 2 differed from Group 1 by showing higher counts in pickle than at the start, the only group to show this.

TABLE VI

SALT RESISTANCE (SURVIVAL) OF MICRO-ORGANISMS REPRESENTING CONTAMINATION PRIOR TO PICKLING

Morphological group	No. of types group in	Days	5% NaCl				10% NaCl				20% NaCl				30% NaCl				Pickle (29.75% NaCl)			
			Inc.	Stat.	Dec.	Inh.	Inc.	Stat.	Dec.	Inh.	Inc.	Stat.	Dec.	Inh.	Inc.	Stat.	Dec.	Inh.	Inc.	Stat.	Dec.	Inh.
Micrococci, Gram pos.	10	5	10				9		1			4	5	1		4	4	2		9	1	
		10	10				8		1	1		3	4	3		2	5	3		8	2	
Micrococci, Gram neg.	6	5	6				4	2				2	3	1			4	2		1	4	
		10	6				5		1			1	4	1			3	3		1	5	
Micrococci, Gram var.	4	5	4				4				1	1	1	1	1		1	2	1	2		1
		10	4				2	1		1	1		1	2			1	3	1	1	1	1
Rods, Gram pos.	9	5	9				6	1	2			3	6			2	3	4		5	4	
		10	9				5		4			3	4	2		2	2	5		4	5	
Rods, Gram neg.	10	5	10				5	1	2	2	1	2	4	3	1		5	4	1	3	3	3
		10	10				6		1	3		4	3	3		1	2	7	1		5	4
Yeast	1	5	1						1					1				1				1
		10				1				1				1				1				1
Total	40	5	40				28	4	6	2	2	12	19	7	2	6	17	15	3	20	12	5
		10	39			1	26	1	7	6	1	11	16	12		5	13	22	2	14	18	6
Staph. aureus		5	×				×					×					×				×	
		10	×				×						×				×				×	
Esch. coli		5	×				×						×				×				×	
		10	×							×				×				×				×
Achromobacter sp. (No. 44)		5	×				×					×					×			×		
		10	×				×						×				×			×		
Micrococcus sp. (No. 42)		5	×				×				×									×		
		10	×				×					×								×		
Micrococcus sp. (No. 46)		5	×				×						×			×				×		
		10	×				×						×			×					×	
Micrococcus sp. (No. 47)		5	×				×						×			×				×		
		10	×				×						×				×			×		

Inc. = Increase over initial counts of 100% or more.   Dec. = Decrease in count of 50% or more.   Stat. = Within the above limits for increase or decrease.   Inh. = Inhibition. No growth on plates.

Group 3.   Four Gram positive, three Gram negative, and one Gram variable species of micrococci, two Gram positive and one Gram negative rods, which declined in numbers from 5 to 30% sodium chloride solution, but maintained numbers up to 10 days in pickle, though declining after one month.
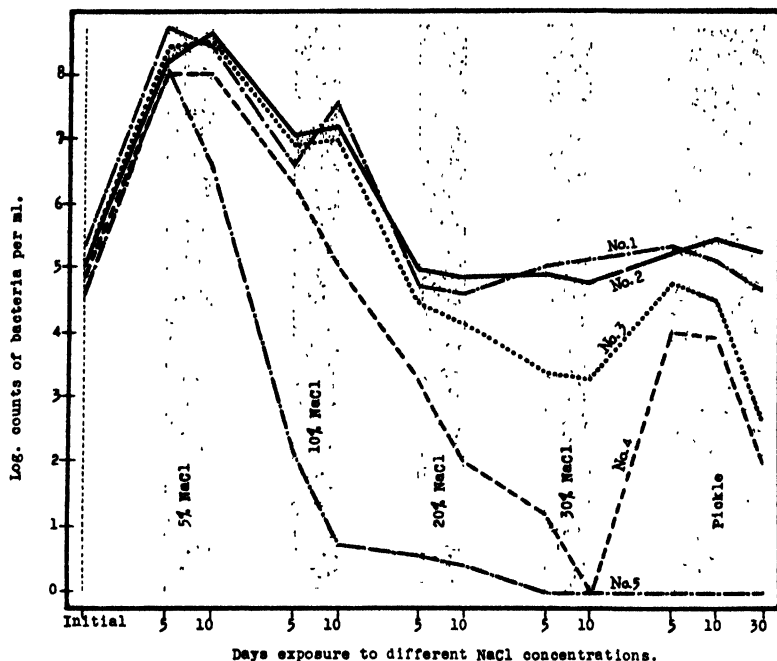
Fig. 3.   *Survival of 46 types of micro-organisms grouped according to their resistance in salt broths of different sodium chloride concentrations and in pickle.*

Group 4.   Fifteen cultures, representing all morphological types with rod forms predominating.   This group showed a noticeable decline in survivors from 5 to 30% sodium chloride, at which latter concentration no species appeared to survive after 10 days.   In pickle of approximately the same concentration however, there was a good measure of survival during the same time.

Group 5.   Four Gram negative rods, together with the one yeast type. These organisms were the most susceptible, declining rapidly in sodium chloride concentrations above 5% and showing no evidence of survival in either 30% sodium chloride or in pickle.

Reactions according to morphological types are given in Table VI, and summarized according to the values "increase", "decrease," "static", or "inhibition", corresponding to standards* set on the basis of the plate counts made after 5 and 10 days respectively.   From the table may be seen the relatively greater resistance of Gram positive micrococci and the comparative

* *For definition of terms see footnote to Table VI.*

susceptibility of Gram negative rods towards higher salt concentrations.   The most striking finding is the much greater resistance displayed in pickle than in salt broths of similar sodium chloride content.   Whereas 15 species were completely inhibited after 5 days, and 22 species after 10 days in 30% sodium chloride broth, but 5 and 6 species respectively were inhibited in pickle of almost identical sodium chloride concentration.   On the other hand, whereas only 5 species showed static counts at the end of 10 days in 30% sodium chloride solution, 16 species showed static or increased counts in pickle, and these comprised 65% of the total number of organisms representing original contamination.   It appears that curing pickle possesses substances that neutralize or mask the toxic or inhibitive action of high salt concentration, despite the fact that it was sterilized at 15 lb. for 30 min., filtered through paper to remove precipitate, and re-sterilized.   Comparing the pickle totals with those for the sodium chloride broths, the inhibitive effect of the former is less than that of 20% sodium chloride broth, approximating that of a solution of one-half its actual salt strength.   That the decreases and inhibitions in the salt broths were not owing to lack of nutrient is borne out by the high increases in the lower salt concentrations.   The effect noted in pickle is "preservative" rather than "stimulative", as seen from the totals in the "static" column (Table VI).   This is further supported by tests of plating media prepared for pickle, which showed no advantage over other media of similar sodium chloride strength.

To examine the possibility that organisms introduced to an environment of 30% sodium chloride might adjust themselves to better growth on higher concentration of salt agar than on the 5% sodium chloride medium used, all cultures that showed inhibition in 30% sodium chloride broth were subjected to a control test in which plating was made on 10 and 15% sodium chloride agar as well as on the regular 5% sodium chloride medium.   In no case did cultures that showed inhibition, as judged by plating on 5% agar, show growth on the 10 or on the 15% sodium chloride agars, permitting the deduction that with the 23 species studied the action of 30% salt involved a toxic effect rather than an adjustment to higher salt concentrations.

It appears that many types of organisms, constituting original contamination of Wiltshire sides, are able to survive the pickling process.   Although the findings do not point to any pronounced activity of such organisms in pickle, either beneficial or deleterious, their ability to survive opens the possibility of their becoming active on sides after pickling, and contributing to storage defects.   The findings therefore point unmistakably to the importance of maintaining packing plants hygienically clean in an effort to reduce this initial contamination to a minimum.

Acknowledgment

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Studies of Fish Spoilage

III. The Trimethylamine Oxide Content of the Muscles of Nova Scotia Fish

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ABSTRACT

Trimethylamine oxide was found in the muscle press juice of all salt water fish examined and in the anadromous fish *Pomolobus pseudoharengus* taken from the sea. Traces were found in *Anguilla* taken from salt water, but none in *Anguilla* from fresh water.

INTRODUCTION

The presence of trimethylamine oxide in dogfish was demonstrated by Suwa (1909). He showed also that bacteria reduce the oxide to trimethylamine and concluded that the particular odour of spoiling sea fish is due to the increase in the amine. Beatty and Gibbons (1937) and Beatty (1938) have shown that, in spoiling cod muscle press juice, trimethylamine is produced only as a result of the action of the bacteria producing the spoilage, and that it is derived from the reduction of trimethylamine oxide.

The literature previous to 1933 has been reviewed by Kutscher and Ackermann (1933). The oxide has been demonstrated by the isolation and analysis of typical salts, in selachians and salt water teleosts, but it was not found in *Anguilla vulgaris, Salmo salar, Perca fluviatilis,* or *Cyprinus carpio.*

Not many quantitative estimations of trimethylamine oxide have been made by the German workers. Kapeler-Adler and Krael (1930) found 0.0167 g. of the oxide and 0.161 g. of trimethylamine per 100 g. of codfish muscle. Since trimethylamine exists in unspoiled fish in very small amounts it is probable that the results obtained by these authors are far from correct. Hoppe Seyler (1933) found 0.3 per cent trimethylamine oxide in the tail muscle of lobster, and 0.05 per cent in crayfish muscle. Cook (1931) analysed muscle tissues of several Canadian fishes, reporting from 0.2 per cent to nearly 0.5 per cent in sea fishes, 0.01 to 0.1 per cent in fresh water fish and anadromous fish. Because of the importance of trimethylamine oxide in studies of fish spoilage, and because of the conflict of evidence as to its occurrence in nature, a survey (not yet completed) has been undertaken as to its occurrence in fresh water and sea fish of Nova Scotia.

ANALYTICAL DATA

Trimethylamine oxide was determined by Lintzel's method (1934). Since the primary purpose of the investigation was to aid in the investigation of fish spoilage, and since previous studies were done mainly on press juice, the trimethylamine oxide content of the muscle press juice rather than of the muscle itself was determined. Analyses of press juice and muscle of *Clupea harengus* showed the press juice to be approximately 19 per cent higher in oxide than the muscle itself. The results of the analyses are shown in table I.

TABLE I. Trimethylamine oxide content of the muscle press juice of Nova Scotia fish.

	Number examined	Trimethylamine oxide (per cent)		
		Maximum	Minimum	Average
SALT WATER SPECIES				
TELEOSTS				
Gadus callarias (cod)	50	0.78	0.59	0.67
Melanogrammus aeglefinus (haddock)	8	0.43	0.34	0.38
Pollachius virens (pollock) . . .	7	0.50	0.44	0.48
Urophycis sp. (hake)	5	0.98	0.78	0.89
Clupea harengus (herring) . . .	8	0.59	0.44	0.48
Scomber scombrus (mackerel) . .	10	0.29	0.22	0.26
Pseudopleuronectes americanus (flounder)	6	0.54	0.25	0.38
Limanda ferruginea (dab) . . .	1			0.418
Glyptocephalus cynoglossus (witch) .	6	0.56	0.23	0.31
ELASMOBRANCHS				
Squalus acanthias (dogfish) . . .	6	1.59	1.25	1.43
Raja laevis (barndoor skate) . . .	1			1.25
Raja senta (smooth skate) . . .	1			0.53
Raja scabrata (prickly skate) . .	2			0.27
FRESH WATER SPECIES				
Perca flavescens (yellow perch) . .	5	0.00		
Ameiurus nebulosus (catfish) . . .	6	0.00		
Catostomus commersonii (sucker) .	2	0.00		
FRESH AND SALT WATER SPECIES				
Anguilla rostrata (eel)				
From fresh water	3	0.00		
" brackish water	3	0.001	0.005	0.004
Pomolobus pseudoharengus (gaspereau) from salt water . .	3	0.28	0.20	0.25

DISCUSSION

The table brings out the following facts. Muscles of all fish examined, that live constantly in sea water, contain trimethylamine oxide in appreciable quantities. *Pomolobus pseudoharengus,* which may live in the sea, whence it migrates into fresh water to spawn, likewise contains the oxide while in salt water and about to enter fresh water. The oxide was not found in muscle of fresh water fish nor in eels while in fresh water. The traces in the muscle of eels taken in salt water may be due to that ingested in food.

As to any physiological action of the trimethylamine oxide, the fact that *Anguilla* taken from the sea contains practically no oxide, shows that it is not necessary to all life in salt water. The wide difference in the values found for *Squalus acanthias* and *Raja scabrata,* both Elasmobranchs and living in salt water, would indicate that if the concentration in the muscles is any indication of that found in the circulating fluids, the oxide is not an important substance in the maintenance of osmotic equilibrium.

The fact that *Anguilla* in salt water does contain traces suggests that food may have some bearing on the concentration of the oxide in the muscles, but the fact that *Squalus acanthias* is about 1,500 times higher in oxide than *Anguilla* would suggest either that the mechanisms of elimination are widely different or that food has only a minor influence on the concentration in the muscles.

Grollman (1929) showed that the urine of *Lophius piscatorius* is high in the oxide. It is very improbable that this compound is used as a means of nitrogen elimination.

As for the theory that trimethylamine oxide acts as an oxygen donor, the data are not sufficient to draw any conclusions.

The marked difference between sea fish and fresh water fish points possibly to a different mechanism of spoilage. Trimethylamine is one of the earliest compounds produced during the spoilage of sea fish, and is definitely responsible for some of the spoilage odour. Previously, the development of spoilage, as determined by the production of trimethylamine, was followed only in members of the cod family. Since the oxide is so widely distributed in sea fish, its reduction by spoilage bacteria should be a general reaction, although the great differences in the oxide contents of various species may mean that different spoilage threshold values may be necessary.

SUMMARY

Muscles of all sea fish examined contained trimethylamine oxide.

Trimethylamine oxide was not found in muscles of fresh water fish.

The oxide was present in muscle of *Pomolobus pseudoharengus* that migrates into fresh water to spawn, but was absent in muscle of *Anguilla* while in fresh water.

The data throw no light on the physiological importance of trimethylamine oxide.

The production of trimethylamine by spoilage bacteria is likely to be a general phenomenon during the spoilage of all sea fishes examined.

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Studies of Fish Spoilage

IV. The Bacterial Reduction of Trimethylamine Oxide*

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ABSTRACT

In fish muscle press juice simulating the surface and the interior of muscle, there is an aerobic environment in the surface layer and an anaerobic environment in the body of the liquid.   The Eh potential of the former is about 0.3 volts and of the latter from -0.5 to -0.10 volt.

It is found that the bacterial population proliferating at 2°C. is chiefly *Achromobacter*, which can be divided into two groups, obligate aerobes and facultative anaerobes. Only the latter group, which is capable of growth in the interior or surface, is responsible for the reduction of trimethylamine oxide with the evolution of trimethylamine. Since the initial total count is made up of a large number of obligate aerobes or non-oxide reducers it is obvious that the total bacterial population cannot be related to trimethylamine production. The appearance of this base therefore may be taken to indicate a bacterial population which is in excess of that responsible for its production.

Molecular oxygen at surface exercises a trimethylamine oxide sparing effect. In practice, however, this effect is not significant from the point of view of the freshness test in the sense of Beatty and Gibbons.

The importance of trimethylamine estimation as an effective method for determination of the freshness of fish has been demonstrated by Beatty and Gibbons (1937).   They found that bacterial increase coincided with the increase in total volatile base and from this deduced that the trimethylamine contribution to the total was directly proportional to the increase in bacteria, as a result of reduction of trimethylamine oxide.

Labrie and Gibbons (1937) advanced a theory accounting for the increase in trimethylamine by a reduction of trimethylamine oxide, initiated by a reduction potential set up by the bacteria when their numbers have reached between 10 and 20 million per ml.

Beatty (1938) has definitely established the precursor of trimethylamine, reporting that even in advanced spoilage of cod muscle press juice it is derived entirely from trimethylamine oxide.   The oxide has been found in all fish examined that live constantly in sea water, codfish muscle averaging 0.67 per cent (Beatty 1939).

It having been shown that reduction of trimethylamine oxide is a result of bacterial action, study of the subject at the Atlantic Fisheries Experimental Station has been continued with the purpose of investigating the role of bacteria in the reduction of this compound.

The investigation has included:

(1) A consideration of bacterial environments simulating the surface and interior of fish muscle.

(2) The development of a technique for separation of a group or groups of bacteria responsible for reduction of trimethylamine oxide.

(3) The determination of a relationship between reduction of the oxide and increase in the bacterial population of these groups.

(4) A study of the role of trimethylamine oxide in the metabolism of the bacteria concerned with the reduction.

The first three points of the programme are dealt with in the present paper; the fourth is presented in a following paper.

BACTERIAL ENVIRONMENTS IN FISH MUSCLE

Brooks (1929, 1936) has pointed out that in untreated meats and bacon the muscles after rigor retain indefinitely some of their respiratory activity; thus when they are exposed to air, dissolved oxygen is present only in a superficial layer a few millimetres thick; below this layer the tissue is completely devoid of oxygen. It is logical to assume that fish muscle behaves similarly and that in the centre or directly below the surface there exists an anaerobic state in the bacteriological sense.

To test the validity of the above assumption a method has been employed which depends upon the reversible oxidizable substances and the residual respiratory systems present in such complex substrates as muscle. Because of the influence of oxygen on these systems the *reduction potential* or Eh in the sense of Hewitt (1936) serves as an index to the degree of aerobiosis and anaerobiosis. Thus Brooks (1938) has shown that in the absence of molecular oxygen the reduction potential of muscle tends to drop to a potential of Eh -2.0. Hewitt (1936) reported the attainment of such low potentials in ground tissue.

As pointed out by Labrie and Gibbons (1937) muscle is not suitable for accurate bacteriological and chemical analysis. In the present investigation muscle press juice has been employed throughout. It must be understood that conditions obtained by employing the muscle juice are not entirely comparable to whole muscle. It is suggested, however, that in the following arrangements employing muscle press juice, conditions were sufficiently comparable to muscle for the results to be significant when deducing the probable course of events during fish spoilage. The juice was obtained by pressing ground pre-rigor codfish muscle, the temperature being kept at about 5°C. One litre of juice was collected within an hour.

Conditions simulating the surface of fish muscle were secured by placing 25 ml. of press juice in a 500 ml. Erlenmeyer flask. The arrangement is illustrated in figure 1. The flask has three openings in the side; in two of these openings were

placed platinum electrodes; the third opening holds the agar-KCl bridge. The platinum electrodes were made of platinum foil 1 cm². These electrodes were carefully prepared before each run, by a cleaning in chromic acid solution and a final electrolysis in a sulphuric acid solution. The electrodes were checked in a standard solution of ferric and stannous chlorides. The platinum foil was placed about 2 mm. below the surface of the muscle press juice. Contact between the calomel cell and the agar bridge was made by dipping the calomel cell into small receptacles containing KCl, fastened permanently to the agar bridges.

Conditions simulating the interior of the muscle were obtained by completely filling stoppered 50 ml. flasks as shown in figure 1. One platinum electrode was placed in each flask, and the agar bridge was similar to the arrangement described above. Each run consisted of four such flasks.

The *potentials* of the surface and of the interior were determined, using a

FIGURE 1. Apparatus for the measurement of redox potentials in muscle juice under conditions simulating the surface and interior of muscle.

vacuum tube potentiometer, which enabled readings to be taken without the occurrence of polarization. The flasks were incubated, and the determinations made, at 2°C.

The potential time curves obtained from measurement at the surface and in the interior of the muscle juice are shown in figure 2. The potentials recorded at four electrodes at the surface and three electrodes in the interior are plotted to show the agreement among the electrodes. Only the first 48 hours as recorded in the graphs are now under consideration.

It is evident that muscle juice is heavily poised in the presence of molecular oxygen; in other words, muscle press juice contains reversible oxidizable substances, which when oxidized by molecular oxygen exert a reduction potential in the order of Eh 0.3 volt. If the supply of molecular oxygen is cut off, the potential falls off rapidly to a negative value. It is obvious that the residual respiratory mechanism of Brooks (1936) was exerting an influence on the reduction

potential through oxygen uptake and subsequent reduction of these reversible systems. From the variation between electrodes for the first 24 to 48 hours, it is evident that the system becomes weakly poised in the absence of oxygen; as the reduction proceeds, the system poises itself in the order of Eh -0.08 volt after 48 hours of incubation. This is nearly comparable to that recorded by Brooks (1938) in the interior of meats. This rapid drop in potential does not coincide with bacterial activity, and it may be concluded that fish muscle is comparable to other tissues in its reducing characteristics. Hewitt (1936) has demonstrated
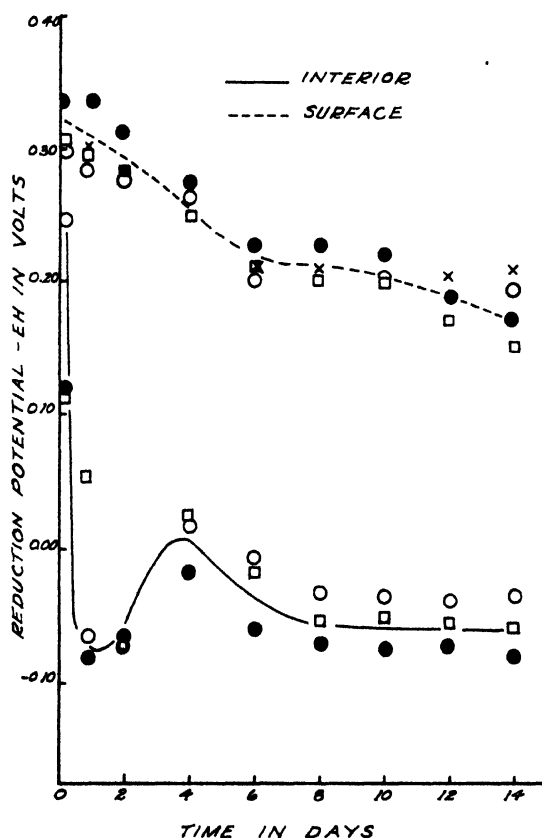
FIGURE 2. Time: potential curves as measured at the surface and in the interior of muscle press juice at 2°C.

a similar effect in serum and attributes the poising to the influence of the oxygen carrying effect of sulphydryl groups present in proteins, auto-oxidizable lipins and haematin compounds.

It may properly be inferred from these results that fish muscle—from a bacteriological point of view—presents two bacterial environments, namely, aerobic and anaerobic. Throughout this investigation, therefore, an attempt has been made to maintain conditions simulating both the surface and the interior of fish muscle, employing muscle juice in the manner described in the present experiment.

SEPARATION OF BACTERIAL GROUPS

It will be shown later that the total bacterial population in the thin layers is greatly in excess of that of the deep layers. If, as was assumed by Beatty and Gibbons (1937), the reduction of trimethylamine oxide is proportional to the total number of organisms, the amount reduced in the shallow layer should be greatly in excess. But since the rate of reduction is approximately the same, it follows that it depends not on the total population but on the types of bacteria present. Thus, before it was possible to correlate bacterial growth with trimethylamine oxide reduction it was necessary to develop a method for the determination of the group or groups of bacteria responsible for the reduction.

PRINCIPLE AND METHOD OF SEPARATION

It was found that certain bacteria growing in muscle press juice can grow anaerobically in the presence of trimethylamine oxide and an oxidizable substrate. All organisms growing on the anaerobic plates were tested aerobically and grew well, that is, they were able to utilize both trimethylamine oxide and oxygen as hydrogen acceptors. Using Spray's (1936) anaerobic plating medium it was not possible to demonstrate obligate anaerobic bacteria, although Shewan (1938) isolated a few species from haddock feces. Hence anaerobic counts, which are described later, represented the facultative anaerobes, and the difference between aerobic counts and anaerobic counts, the obligate aerobes.

PREPARATION OF MEDIA AND COUNTING TECHNIQUE

With the reduction of the oxide there is a liberation of the base, trimethylamine. Thus, in unbuffered media containing trimethylamine oxide, a slight reduction of the oxide may immediately place the reaction of the medium out of the physiological range. This point is clearly demonstrated in figure 3, in which it will be seen that beef extract broth, being poorly buffered, undergoes a rapid shift in reaction on the addition of even small amounts of alkali. On the other hand, cod muscle press juice is highly buffered, as will be observed. As an index to the degree of buffering, the media employed have been buffered to the same extent as cod muscle press juice by the addition of phosphates; a comparison between the buffering capacity of press juice and the buffered medium is shown in figure 3.

Throughout the investigation strict control of hydrogen-ion concentration and of buffering capacity of the medium was maintained; the determinations were made with the aid of a glass electrode. The medium—the buffering curve of which is shown in figure 3—was prepared as follows: Beef extract 3.0 g., peptone 5.0 g., sodium chloride 5.0 g., potassium phosphate (KH_2PO_4) 6.8 g., sodium phosphate ($Na_2HPO_4.12H_2O$) 18.0 g., agar 15.0 g., dissolved in water, adjusted to pH 6.9 with sodium hydroxide, and made up to 1 litre.

This medium was used for all the aerobic counts; the anaerobic counts were made on the same medium plus 7.5 g. trimethylamine oxide per litre, which is a concentration of the order found in cod muscle press juice. The medium was placed in test tubes which contained approximately 11 ml. During the auto-

claving there was a slight precipitate of insoluble phosphate, but by using individual tubes each plate contained the same amount of precipitate as well as the same volume of medium. The precipitate was crystalline and easily distinguishable from bacterial colonies when magnified 14 diam.

The dilution fluid was also buffered and prepared as follows: Sodium chloride 10.0 g., potassium phosphate (KH_2PO_4) 4.5 g., sodium phosphate ($Na_2HPO_4.12H_2O$) 12.0 g., dissolved in water, adjusted to pH 6.9 with sodium hydroxide, and made up to 1 litre.

The counting technique was based on that of Jennison (1937). Duplicate dilutions and triplicate plates from each dilution were employed for each count; nine ml. of the above dilution fluid were placed in individual test tubes; sterilized
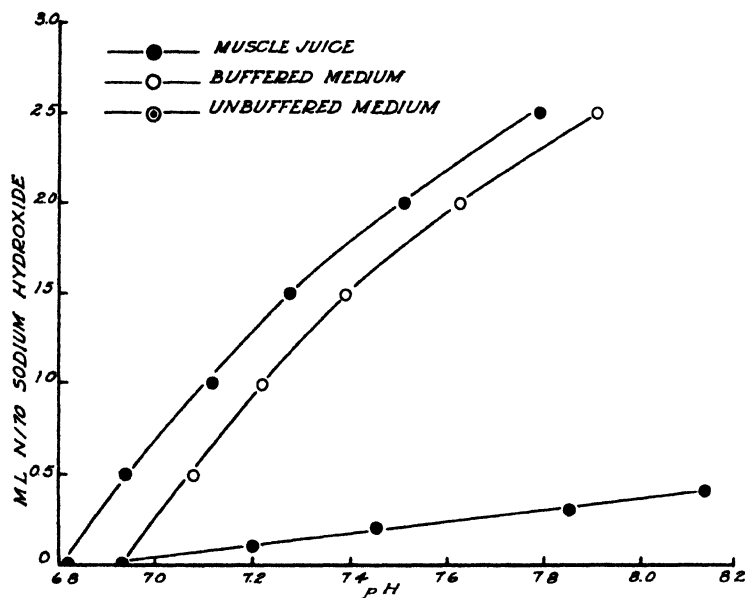
FIGURE 3. The buffering capacity of unbuffered and buffered media as compared to muscle press juice.

corks were used to plug the tubes during mixing. The smaller volumes require a greater number of dilution blanks for each count but assure an even distribution of organisms. Counting conditions were maintained as uniformly as possible.

All plates were incubated at 25°C. and counted after four days. The problem presented by the necessity of making anaerobic counts in large numbers was solved by the designing of an economical and controlled anaerobic chamber which is described in a subsequent paper.

BACTERIAL RELATIONSHIP TO REDUCTION OF TRIMETHYLAMINE OXIDE

METHODS

The reduction of trimethylamine oxide was determined and recorded as the increase in trimethylamine nitrogen. Values above 40 mg. per cent were deter-

mined, employing the Beatty and Gibbons (1937) modification of the Parnas-Mozolowski apparatus. The initial and lower values were determined by the Beatty and Gibbons (1937) adaptation of the Conway and Byrne (1933) method for the measurement of ammonia.

A further modification of the Conway and Byrne method for the determination of trimethylamine has been made, because of the difficulty in foretelling the necessary quantity of standard acid as employed in the Conway apparatus, especially during the logarithmic increase in trimethylamine. This difficulty was largely overcome by substituting boric acid for the standard acid. With this modification it was possible to cover a range from 0.0 to 40.0 mg. per cent nitrogen employing two boric acid solutions. The method had a further advantage in that the boric acid could be measured with a pipette; the accuracy of this measurement was not important. The required strengths of boric acid solutions were determined by means of titration curves; it was found that trimethylamine was trapped until the hydrogen-ion concentration approached pH 8.0. From these curves it was possible to determine the minimum strength of boric acid necessary to trap a definite quantity of trimethylamine. Moreover it was necessary to keep the concentration of boric acid at a minimum since the buffering tended to decrease the sensitivity of the indicator. In measuring from 0 to 10 mg. per cent nitrogen 1 ml. of 0.1 M. solution of boric acid was used, and from 10 to 40 mg. per cent nitrogen 1 ml. of 0.3 M. solution. The solutions were made up in 250 ml. quantities; in making up this amount 10 ml. of Conway and Byrne's (1933) modification of Tashiro's methyl red methylene blue indicator was added, plus sufficient alkali to bring the indicator to the neutral point; the solution was finally brought up to volume. It was necessary to prepare fresh boric acid solutions at least every two weeks, since the indicator deteriorated, making the end point difficult to detect. The back titrations were carried out in the usual manner, using micro-burettes containing standard acid. It was necessary to employ two acids, one for low trimethylamine values and a stronger solution for the high values. The accuracy of the method was tested on pure solutions of trimethylamine; the base could be recovered within 1.0 per cent.

EXPERIMENTAL

Surface conditions were obtained by placing 25 ml. of muscle press juice in each of a number of 500 ml. Erlenmeyer flasks (depth 4 mm.) plugged with cotton in the conventional manner. Conditions simulating the interior were obtained by filling 50 ml. flasks and stoppering tightly. A sufficient number of flasks were prepared so that the whole contents of each flask could be used for bacteriological and chemical analysis; this technique prevented error due to sampling. It was found important to mix the muscle juice thoroughly to assure an even distribution of bacteria. The flasks were incubated at 2°C.; individual flasks were removed at intervals of two days for analysis.

The results are given in figure 4. On examining the curves the outstanding observation is the influence of the environments. The trimethylamine curves show that the availability of molecular oxygen at the surface resulted in a trimethylamine oxide *sparing* effect. In this respect the effect was most evident for

the first four days of incubation, after which the reduction of the oxide at the surface was nearly comparable to that of the interior. The phenomenon will be explained later.

On examining the curves of the total aerobic population and comparing them with the trimethylamine curves, it will be noticed that the number of bacteria decreased for the first two days, while the trimethylamine increased over the same period; this was not always true as shown from repeated experiments, but in no case was there a relationship between the total count and trimethylamine increase over this period. From a consideration of the area between these curves it may be concluded that a group of organisms dependent upon molecular oxygen were proliferating at the surface.
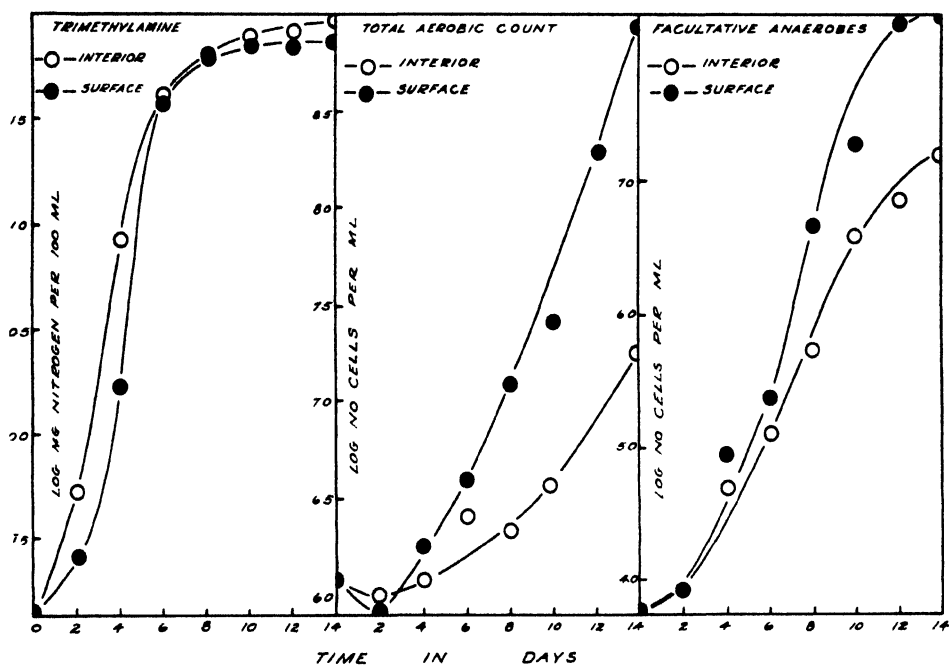
FIGURE 4. Relationship between trimethylamine increase, total aerobic population and facultative anaerobes as determined at the surface and interior of cod muscle press juice at 2°C.

On comparing the facultative anaerobic curves with the trimethylamine curves there is evidence of a close relationship. It is evident that the numbers were greater at the surface since they were able to take advantage of the molecular oxygen present, while those in the interior had to rely upon hydrogen acceptors other than molecular oxygen.

If the data used for the facultative anaerobic curve of the interior are subtracted from the data for the total aerobic count of the interior, the resultant curve will actually show a decrease in number, which indicates that obligate aerobes were unable to grow in this environment. If the same procedure is applied to the surface curves, the resultant curve will show a rapid increase in

numbers, indicating the rapid proliferation of obligate aerobes at the surface. This point is effectively illustrated in figure 5 where the obligate aerobic numbers were obtained and plotted in this manner. These obligate aerobes made up a large portion of the initial total population which therefore cannot be related to the evolution of trimethylamine, as was done by Beatty and Gibbons (1937) and Labrie and Gibbons (1937) before this differentiation was possible.

INFLUENCE OF AERATION

In repeated experiments it was possible to increase the area between the trimethylamine curves plotted in figure 4, by decreasing the depth of the surface layer. It was assumed that the availability of molecular oxygen was increased, exerting an oxide *sparing effect*.

To magnify this effect and thus confirm the assumption, the above experiment, the results of which are plotted in figure 4, was repeated, determining the influence of strong aeration on the reduction of trimethylamine oxide. To increase the aeration, a stream of air with sufficient force to keep the thin layer of muscle juice in continual movement was passed over the surface.

Facultative anaerobic and obligate aerobic bacterial counts were determined as described in the previous experiments. In view of the hypothesis of Labrie and Gibbons (1937) oxidation-reduction potentials were measured in the same manner as described in the first section of this paper.

The results of the first six days of the experiment have been plotted in figure 5, since the oxide *sparing effect* was most evident in this period. The "aerated data" and "non-aerated data" are from the results of two different experiments, using different muscle juice; they were controlled by running parallel experiments under conditions imitating the interior of muscle. The results confirm the previous assumption, aeration increasing the area between the trimethylamine curves of the aerated experiment and the control. The extreme aeration prevented the reduction of trimethylamine oxide for the first 48 hours. When the bacteria began to proliferate rapidly, as shown from the facultative anaerobic curves, the oxide was reduced regardless of the aeration. The bacterial growth curves were not influenced significantly for the first six days; on following days, the obligate aerobes at the aerated surface proliferated rapidly as compared to those at the non-aerated surface. The oxidation-reduction potential curves confirm those plotted in figure 2; these curves were not altered significantly by the aeration. As shown in figure 5, the surface electrodes were poised at Eh 0.3 volt; active proliferation produced a drop to the order of Eh 0.2 volt. The potential as recorded at the electrode in the muscle juice simulating the interior shows the same reduced environment as recorded in figure 2. The characteristic trend as recorded at these electrodes between 2 and 6 days has been evident in all experiments; it is probably indicative of the oxidation or reduction of a system having a characteristic potential within this range.

It is evident that fish muscle press juice is heavily poised and therefore the potential as measured at a platinum electrode is no criterion of the potential induced at cell surfaces. Yudkin (1935) came to the same conclusion employing cell suspensions and weakly poised solutions. The fact is that during the loga-

rithmic growth phase, the facultative anaerobic *Achromobacter* were able to set up the required reducing intensities at their cell surfaces for the activation of trimethylamine oxide as hydrogen acceptor, while at the same time, the potentials
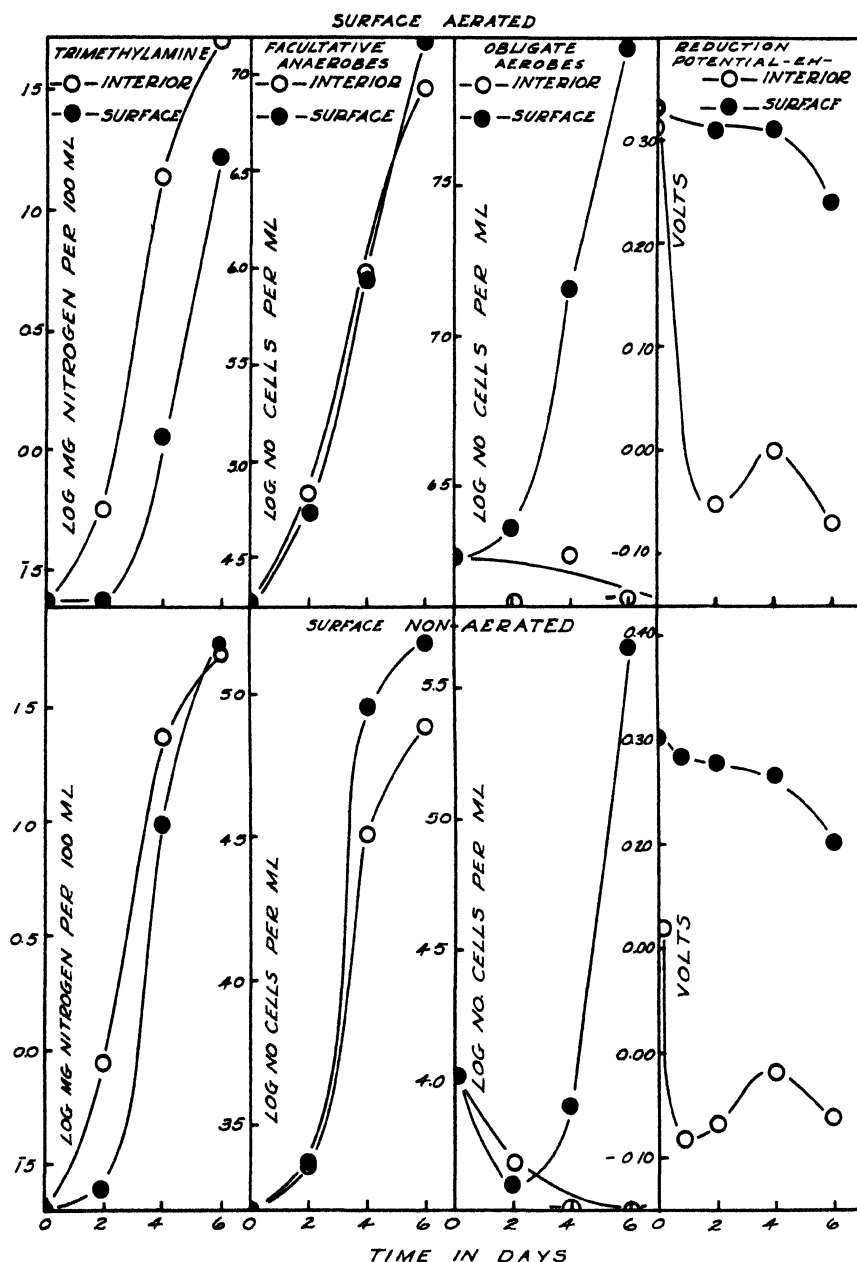
FIGURE 5.   Influence of aeration on the trimethylamine increase, obligate aerobes, facultative anaerobes and reduction potentials at the surface of cod muscle juice at 2°C. as controlled by similar measurements in the interior.

recorded at the electrode indicated that their environment was poised at a relatively high potential of Eh 0.2 to 0.3 volt. The hypothesis of Labrie and Gibbons (1937) on the reduction of trimethylamine oxide assuming the attainment of a critical potential of the environment must be referred, not to the conditions of the media, but to the surface of the cell where the trimethylamine oxide has a metabolic significance.

DISCUSSION OF "SPARING EFFECT"

Stephenson (1930) has pointed out that facultative anaerobes can effect their oxidations more profitably from molecular oxygen than through anaerobic oxidations by hydrogen acceptors. This was observed when the reduction of trimethylamine oxide was prevented by intense aeration during the first 48 hours when the bacterial increase was not great. When the numbers greatly increased even this failed to stop the reduction of the oxide, for as Rahn (1932) and Anderson (1938) have pointed out, the solubility of oxygen is small and it soon became insufficient to support increasingly large numbers. This led to the utilization of the hydrogen acceptors which were in solution.

This *sparing effect* as applied to fish muscle surfaces would not be significant. As already shown there are many bacteria at the surface which depend entirely on molecular oxygen for their oxidations, setting up a competition for the dissolved oxygen present. The aeration necessary to make the phenomenon significant would never occur at muscle surfaces; and would have no attenuating influence on the reduction of trimethylamine oxide. Beatty and Gibbons (1937) have shown that the rate of trimethylamine production was greater at surface tissues such as gills and peritoneum than in muscle tissues. This can be explained by the difference in contamination between the two areas and the inefficiency of the transport of oxygen to the cells as described above.

THE ORGANISMS CONCERNED

No extensive systematic study has been made of the facultative anaerobes or the obligate aerobes except that required to place them in their respective genera. Some two hundred species that reduce trimethylamine oxide were studied. The majority of the species in this group were members of the genus *Achromobacter*, although there were a few species of lactose splitting types. The obligate aerobic types belonged to the *Flavobacter* and *Achromobacter*; after 4 or 5 days of incubation at 2°C., species present in muscle juice other than *Achromobacter* were not significant. It may be concluded, therefore, that the genus *Achromobacter* is chiefly responsible for the spoilage of cod muscle at low temperatures. These results are in agreement with those of other authors. Gibbons(1934) and Stewart (1932) have made systematic surveys of bacteria associated with spoiling fish and found members of the genus *Achromobacter* most abundant.

Throughout the remainder of the investigation the *Achromobacter* have been divided into the groups depending on their ability to reduce trimethylamine oxide,—(a) *reducing Achromobacter* and (b) *non-reducing Achromobacter*. There is, however, the possibility of either group becoming adapted to the other condition.

As previously stated, obligate anaerobes play no part in the initial stages of fish spoilage.

A few species of microaerophilic, acidogenic strains were isolated; but they were never present after the initial count. Studies revealed that they were unable to reduce trimethylamine oxide and indicated their inability to proliferate at 2°C. They appear to be typical of the strains isolated by Shewan (1936), and the *Lactobacilli* of Watson (1938).

REDUCTION BY PURE CULTURES

It is evident (figure 4) that there is a relationship between the growth of mixed cultures of facultative anaerobes and the production of trimethylamine, as is also the lack of such in the growth of mixed culture of obligate aerobes. This was further examined by using pure cultures prepared from the same material.

Separate strains were selected from mixed cultures appearing in the plates used for the 8 and the 12 day counts, in figure 4. These were inoculated on to media containing trimethylamine oxide equivalent to 40 mg. nitrogen per 100 ml. of medium. Those strains capable of anaerobic growth in the previous experiment successfully reduced the oxide, while those not capable of anaerobic growth failed to do so.

REDUCTION RELATED TO GROWTH OF A PURE STRAIN

The ordinary stock medium minus the agar with the addition of trimethylamine oxide equivalent to 70 mg. nitrogen per 100 ml. was placed in 10 ml. lots in a series of test tubes. A tube of the above medium was inoculated from a test culture of a typical member of the facultative anaerobe group as isolated above (reducing *Achromobacter*) and incubated for 12 hours at 25°C. This sub-culture was thoroughly mixed and 0.1 ml. was placed in each of the above tubes. After the cultures were mixed, they were incubated at 25°C.; tubes were removed at two hour intervals for analysis; total counts and trimethylamine determinations were made.

The results are expressed graphically in figure 6. It will be observed that the reduction of the oxide closely paralleled the growth of the organism.

REDUCTION OF TRIMETHYLAMINE OXIDE AND NITRATES CORRELATED

Hess (1934) has demonstrated the ability of *Achromobacter* to adapt its metabolism to the reduction of nitrates at low temperatures; in the light of the findings of Quastel, Stephenson, and Whetham (1925) these bacteria were carrying on respiration at these low temperatures employing nitrates as hydrogen acceptors. If this conclusion is correct, additional significance would be added to the trimethylamine test of Beatty and Gibbons (1937) when it is considered that Hess observed this adaptation to the rapid reduction of nitrates at temperatures in the order of 0° to −3°C.

Buffered stock medium, made semi-solid by the addition of 1.5 g. of agar per litre, plus 0.1 per cent potassium nitrate, was used for the nitrate reduction tests. The oxide medium was prepared from the stock medium as described above, but

with the addition of trimethylamine oxide equivalent to 70 mg. per cent nitrogen. Nitrate reduction was determined by a qualitative test for nitrites and the oxide reduction by quantitative determinations for trimethylamine. The organisms were secured from a 14 day count on cod muscle juice formerly incubated at 2°C' The cultures were incubated at 25°C. for 48 hours before testing.

The results are set forth in table I. It is evident that all *Achromobacter* capable of the reduction of trimethylamine oxide also reduced nitrates; the species not reducing nitrates cannot reduce the oxide. It is interesting to note that the *Flavobacter* studied were able to reduce nitrates but not the oxide.
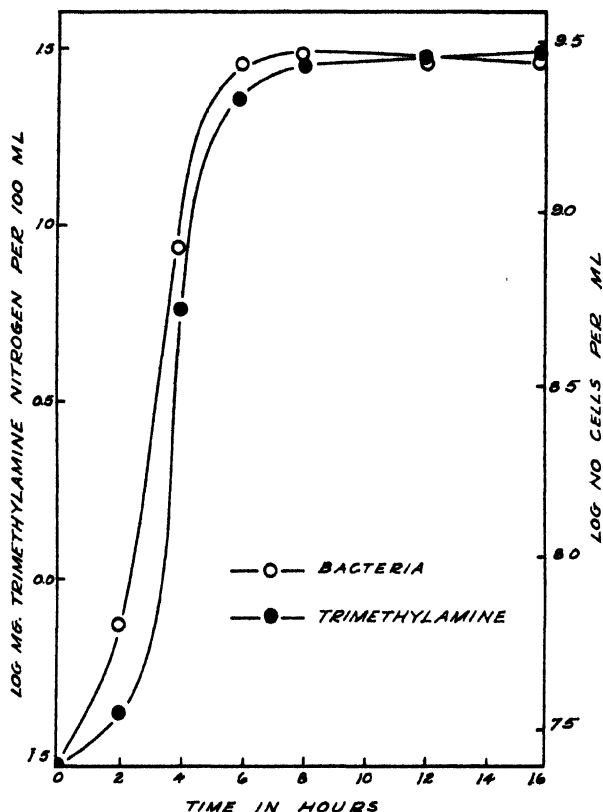
FIGURE 6. The relationship between trimethylamine oxide reduction and the bacterial increase of a pure strain of *reducing Achromobacter* at 25°C.

TABLE I. The relationship between the reduction of trimethylamine oxide and that of nitrates by bacteria isolated from cod muscle press juice.

Bacteria represented	NO_3 reduction (nitrite)	$(CH_3)_3NO$ reduction (mg. nitrogen)
50×10⁶ (*non-reducing Achrom.*)....	—	0.48
50×10⁶ (*reducing Achrom.*)........	+	68.0
10×10⁶ (*Flavobacter*).............	+	0.22
Control........................	—	0.50

SUMMARY

Oxidation-reduction potentials have been determined in cod muscle press juice under conditions simulating the surface and the interior of the muscle. The Eh potential of the surface is in the order of 0.3 volt and that of the interior is −0.05 to 0.10 volt. These results indicate the existence of an aerobic and anaerobic environment in the bacteriological sense.

Boric acid has been substituted for standard acid in the method of Conway and Byrne; this enables a rapid and convenient method for the determination of trimethylamine.

The reduction of trimethylamine oxide during the spoilage of cod muscle press juice at 2°C. is not a function of the total bacterial population. The bacteria are divided into *non-reducing Achromobacter* and *reducing Achromobacter*. The former group are obligate aerobes and proliferate only at the surface in the presence of molecular oxygen; the latter group are facultative anaerobes and grow at the surface and in the anaerobic interior at the expense of hydrogen acceptors, excluding trimethylamine oxide. Obligate anaerobes and species other than *Achromobacter* are not significant in the initial spoilage period.

There is no direct correlation between the reduction potential of the environment external to the cell and the reduction of trimethylamine oxide.

Oxygen present at the surface produces an oxide "sparing effect" which is increased on aeration. The significance of the phenomenon is discussed.

The reduction of nitrate as a hydrogen acceptor by the *reducing Achromobacter* is comparable to the reduction of trimethylamine oxide. The importance of this observation is pointed out.

The reduction of trimethylamine oxide by pure cultures of *reducing Achromobacter* under laboratory conditions is reported. The results show a parallel relationship between the reduction of the oxide and the growth of a pure strain.

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Studies of Fish Spoilage

V. The Role of Trimethylamine Oxide in the Respiration of Achromobacter*

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ABSTRACT

A general equation involving the reduction of trimethylamine oxide by *Achromobacter* is derived and tested, and is $AH_2+(CH_3)_3NO \longrightarrow A+(CH_3)_3N+H_2O$, where AH_2 is the hydrogen donator and A the oxidized substrate. The reduction of trimethylamine oxide as hydrogen acceptor with the evolution of trimethylamine is a linear function of time in the presence of cell suspensions and single hydrogen donators including glucose, glycogen, lactate, and pyruvate. All strains of *Achromobacter* are not able to reduce the oxide, although they may contain the same dehydrogenases as revealed by employing the methylene blue technique. Small concentrations of hydrogen acceptors such as nitrate and methylene blue inhibit the reduction of the oxide. Since fumarate is not inhibitive and supports anaerobic growth there is evidence of a preferential activation of hydrogen acceptors.

The present investigation deals with the last point of the programme outlined in the preceding paper (Watson 1939), namely, the role of trimethylamine oxide in the metabolism of the bacteria responsible for its reduction. As described in that paper, a method was developed for differentiating the bacteria proliferating in cod muscle press juice into *reducing* and *non-reducing Achromobacter*, owing to the ability of the former group to grow anaerobically in the presence of trimethylamine oxide and an oxidizable substrate.

The evidence presented in the foregoing paper suggested trimethylamine oxide functioning as a hydrogen acceptor in the anaerobic growth of the reducing *Achromobacter*. Wieland's theory of hydrogen transfer has been extensively applied to bacterial dehydrogenations by Quastel and Whetham (1924, 1925a, 1925b), Quastel, Stephenson and Whetham (1925) and Quastel and Wooldridge (1925). These authors have shown the importance of methylene blue, fumarate, nitrates, chlorates and many other compounds in the role of hydrogen acceptors during the anaerobic respiration of bacteria. Krebs (1937) has made a thorough study of fumarate as hydrogen carrier in the respiration of *B. coli*. He concluded that fumarate, pyruvate, oxaloacetate and probably carbon dioxide act as respiratory catalysts.

267

In order to determine the role of trimethylamine oxide in the respiration of *Achromobacter* there were included in this investigation: (1) the measurement of the ability of cell suspensions of both *reducing* and *non-reducing Achromobacter*, as defined in the previous paper, to reduce hydrogen acceptors including trimethylamine oxide in the presence of different hydrogen donators; (2) the determination of the influence of various hydrogen acceptors on the reduction of trimethylamine oxide; (3) a quantitative investigation of the coupled oxidation-reduction reaction between lactic acid as hydrogen donator and trimethylamine oxide as the acceptor in the presence of *reducing Achromobacter*.

THE REDUCTION OF HYDROGEN ACCEPTORS

WITH DIFFERENT SUBSTRATES

It was planned to investigate the reduction of the oxide employing cell suspensions of the organisms in conjunction with pure substrates for hydrogen donators. The two groups of *Achromobacter* each include many species. In the present study *reducing Achromobacter* and *non-reducing Achromobacter* are used to designate single pure strains typical of these groups; the same strains have been employed throughout the investigation.

The advantages of the cell suspension technique have been adequately outlined by Wilson (1938).

The stock medium plus trimethylamine oxide (Watson 1939) placed in Kolle flasks was employed to grow the organisms. After 24 hours' incubation the growth was washed off with a buffered saline solution made similarly to the dilution fluid as described in the previous paper. The cells were washed and centrifuged twice, the suspension of them brought to a definite volume, and the number of cells per ml. of suspension determined; 1 ml. of suspension contained in the order of 65×10^9 cells. The suspensions were stored at 10°C. and generally used within 24 hours after preparation.

The hydrogen donators used were glycogen, glucose and lactic acid made up in buffered saline solution which was finally adjusted to a hydrogen-ion concentration of pH 6.9. These carbohydrates and carbohydrate derivatives were chosen because Sharp (1934, 1935) demonstrated a continuous breakdown of glycogen and accumulation of lactic acid, and Beatty and Collins (unpub.) showed that lactic acid disappears concomitantly with the production of trimethylamine.

In the present and subsequent experiments strict control of hydrogen-ion concentration was maintained; reaction mixtures, cell suspensions and media were adjusted and buffered to a hydrogen-ion concentration of the order of pH 6.9, as determined by the glass electrode.

To test the reduction of the oxide in the presence of these hydrogen donators, six series, each of approximately ten tubes, were made up. Three ml. of trimethylamine oxide solution were added to each tube, the final concentration being 50 mg. nitrogen per 100 ml. of medium. Glucose solution was added to two series making a final concentration of 0.5 per cent; one series contained 1.0 per cent lactic acid; one series was made up to 0.5 per cent glycogen; the remaining series were controls in that no hydrogen donators were added. Five ml. of bac-

terial suspension (*reducing Achromobacter*) were added to each tube in one glucose, the glycogen, and one control series; a similar quantity of *non-reducing Achromobacter* suspension was added to each tube of the remaining series. These tubes were stoppered tightly and incubated at 30°C. A tube from each series was removed every two hours for analysis. The reduction of the oxide was followed by making trimethylamine determinations by the Conway and Byrne method (Watson 1939).
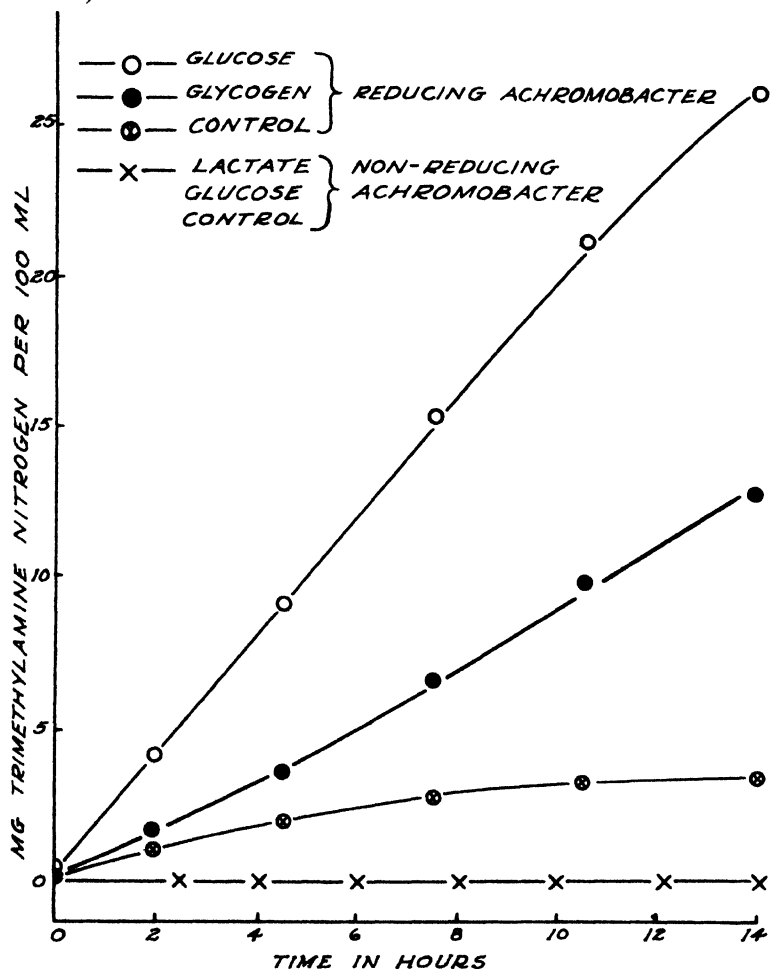
FIGURE 1. Reduction of trimethylamine oxide as hydrogen acceptor by cell suspensions of *Achromobacter* in the presence of different donators.

The results are expressed graphically in figure 1. It will be observed from the curves that the *non-reducing Achromobacter* were unable to reduce the oxide in the presence of the substrates, but the *reducing Achromobacter* did so readily, thus confirming previous observations (Watson 1939). The oxide was reduced as a linear function of time. In the absence of the donator, as shown from the control there was very little reduction of the oxide, that during the first 8 hours

being accounted for by the oxidizable substances present in the suspension. This effect has been reduced in subsequent experiments by aerating the suspension at room temperature and re-centrifuging before making up to volume.

As a Measure of Dehydrogenase Activity

From the results of the above experiment it may be concluded that under the given conditions trimethylamine oxide plays no part in the respiration of *non-reducing Achromobacter*. In view of the possibility of this being due to adaptation (Watson 1939), a comparative study of respiration in these two groups of *Achromobacter* has been undertaken.

The methylene blue technique of Thunberg (Meldrum 1934) has been employed. A modification of the inverted U-tube of Quastel and Whetham (1924) was used as a reaction chamber; this tube will be described later. The bacterial suspension was placed in one arm, and the methylene blue and hydrogen donator in the other. After evacuating the air and refilling with oxygen-free nitrogen, the tube was incubated in a water bath at 30°C. When the temperature became constant the contents of both arms were mixed and the time recorded for complete reduction. The final concentration of methylene blue was 0.001M. and the concentration of each donator was of the order of 0.025M. For the trimethylamine oxide reduction tests, a series of tubes was prepared as described in the preceding experiment. The concentration of the donators was 0.025M. and the final concentration of trimethylamine oxide was equivalent to 8 mg. nitrogen per 100 ml. As in the methylene blue technique, 5 ml. of bacterial suspension were added to each tube. The tubes were incubated at 30°C. and the per cent reduction determined from the trimethylamine values.

The results have been recorded in table I. It is evident that the *reducing Achromobacter* and *non-reducing Achromobacter* contained active dehydrogenases when methylene blue was employed as hydrogen acceptor. On the other hand, trimethylamine oxide was reduced as hydrogen acceptor only by the *reducing Achromobacter*. In considering these results it should be recalled that methylene blue is a reversible system and does not require activation by the cell in the sense of other hydrogen acceptors such as nitrate and fumarate. From these results it is evident that trimethylamine oxide, like nitrate, requires a degree of activation by the cell. Thus, *non-reducing Achromobacter* either have lost the necessary activating power through the influence of their environment or have never possessed it.

TABLE I. Dehydrogenase activity of *Achromobacter* as measured by the reduction of methylene blue and trimethylamine oxide

Donator	Methylene blue (min. for 100% reduction)		Trimethylamine oxide (% reduction in 11 hours)	
	Achromobacter		*Achromobacter*	
	reducing	non-reducing	reducing	non-reducing
Glucose.	50	80	66 0	0
Glycogen	70	113	26 2	0
Lactic acid	80	30	35 5	0
Pyruvic acid	70	60	53 0	0
Control.	∞	∞	2.1	0

As an Index to Oxidation of Hydrogen Donators

The technique varied from the first experiment in that each series was contained in a 50 ml. Erlenmeyer flask. The samples were pipetted for analysis and anaerobic conditions were maintained by passing oxygen-free nitrogen through the mixtures. The final concentration of donators was 0.05 M., while the tri-
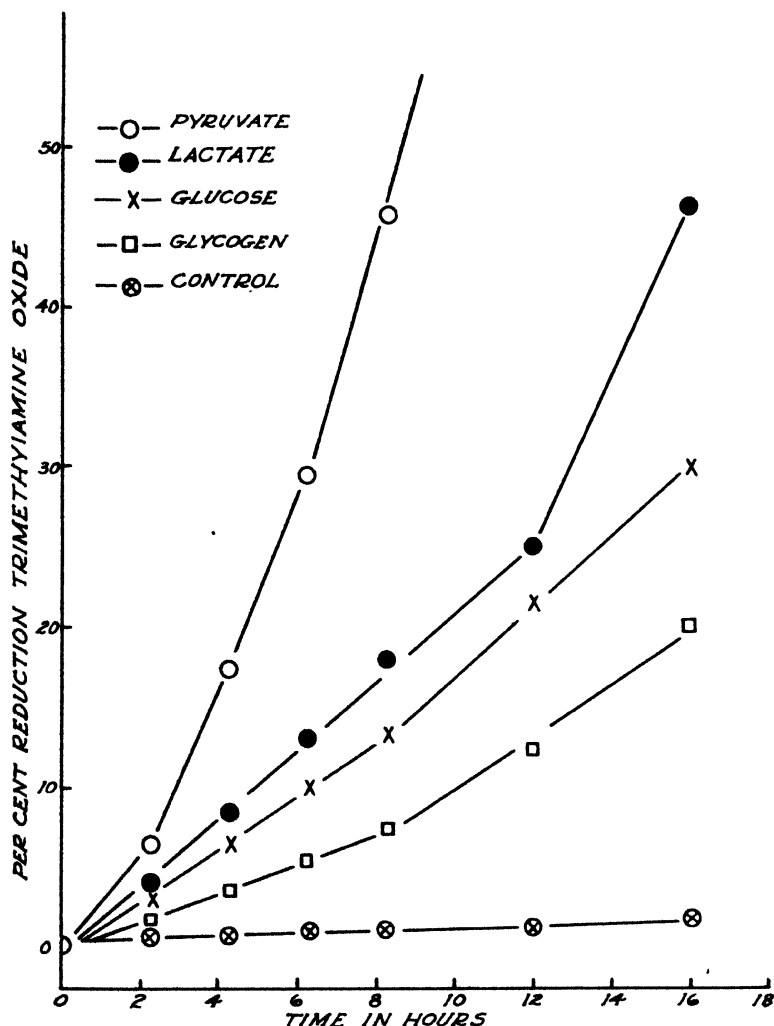
FIGURE 2. Comparative rates of trimethylamine oxide reduction as an index to oxidation of hydrogen donators by a cell suspension of *reducing Achromobacter*.

methylamine oxide content was equivalent to 28.0 mg. nitrogen per 100 ml. One series was maintained as a control in that it contained no hydrogen donator. A bacterial suspension of *reducing Achromobacter* was added to each series so that 1 ml. of the final solution contained 4×10^9 cells. The flasks were incubated in a constant temperature bath at 30°C. The percentage reduction of trimethylamine

oxide was determined by the estimation of trimethylamine at intervals of approximately two hours.

The results are expressed graphically in figure 2. It will be observed that the reduction of the oxide was a linear function of time as seen in figure 1. It is obvious that pyruvate was oxidized more rapidly than lactate. Moreover, from table I it will be noticed that after 11 hours this increase in oxidation of pyruvate was accompanied by an increased reduction of trimethylamine oxide. The relative rates of reduction in the presence of glucose and glycogen and the failure of the oxide to be reduced in the control approximate the results as plotted in figure 1. From the results in figure 2 it will be observed that there are definite changes in rates with time, most evident for the pyruvate curve, and next for the lactate. Krebs (1937) has observed this for pyruvate, employing fumarate as hydrogen acceptor. An explanation has not been advanced.

It is seen in table I that glucose has a higher reducing activity than the other donators, but in figure 2 that both pyruvate and lactate have a higher reducing activity than glucose. The relationship, however, between glucose-glycogen and pyruvate-lactate always remains the same; in other words, glucose displayed more reducing activity than glycogen and pyruvate was oxidized more rapidly than lactate. This discrepancy in dehydrogenase activity may be explained in the light of the adaptive enzyme phenomenon as discussed by Knight (1936) and Anderson (1938).

INTERACTION OF OTHER HYDROGEN ACCEPTORS

The influence of other hydrogen acceptors on the reduction of trimethylamine oxide was determined both by growth experiments and in the presence of cell suspensions.

In the growth experiment four series of tubes were set up each containing 10 ml. of the stock medium of Watson (1939), made semi-solid by the addition of 1.5 g. agar per litre, and with trimethylamine oxide to give a final concentration of 20 mg. nitogen per100 ml. To each of three series methylene blue, nitrate or fumaric acid was added to a final concentration of 0.002 M. and the fourth series kept for control. Individual tubes of each series were inoculated with 0.01 ml. of a 48 hour culture of *reducing Achromobacter*; incubation was carried out at 30°C.

The cell suspension technique was similar to that previously employed except that 50 ml. flasks were used and anaerobic conditions were maintained by passing oxygen-free nitrogen through the solution. Four series were prepared, each containing lactic acid, trimethylamine oxide and bacterial suspension; to each of three series were added small amounts (final concentration 0.002 M.) of methylene blue, nitrate or fumaric acid; and the fourth kept for control.

The results are expressed graphically in figures 3 and 4. Figure 3 shows the influence of the acceptors on the reduction of the oxide during the growth of the organism. In the presence of methylene blue there was an inhibitive effect until the dye was partially reduced at (C); it is important, however, to observe that the methylene blue was not completely reduced until considerable oxide was

reduced at (D). The nitrate produced an inhibiting influence similar to the methylene blue. Spot test for nitrite showed that the nitrate was reduced immediately at (A); tests indicating no further reduction were obtained at (B). On the other hand, fumarate did not exhibit this inhibiting phenomenon, that is, the reduction of the oxide in the fumarate series and in the control series proceeded simultaneously.

The results plotted in figure 4, obtained by utilizing cell suspensions, confirm those of figure 3. Methylene blue completely inhibited the reduction of the oxide for the first 30 minutes of incubation; at this point (C), however, the initial reduction of the methylene blue occurred and the oxide was reduced; complete reduction of the dye was observed at (D). Nitrates were immediately reduced, starting at (A); no further reduction could be observed at (B). Fumarate, as was observed in the growth experiment, followed the control for the first two hours; at this point, the cells activated it to function catalytically. Quastel, Stephenson, and Whetham (1925) have reported fumarate acting not only as a hydrogen acceptor, but also as an oxygen acceptor becoming a carbon source. Thus, it is possible to explain the catalytic action of fumarate in the light of this consideration.

This inhibiting phenomenon was not a result of attenuated growth or activity of the cell. Growth was as prolific in the presence of methylene blue and nitrate as that observed in the control. It should be recalled that the reduction of the oxide occurred before the methylene blue was completely reduced; thus, as concluded by Yudkin (1935) and discussed by Watson (1939), such a phenomenon is not directly related to the potential of the environment but to cell surface activities. The evidence points to a poising influence on these cell activating mechanisms. As discussed by Cannan, Cohen, and Clark (1926) cells deprived of oxygen develop progressively a more negative potential. In this course they traverse in order the zones characteristic of reversible indicators; thus in one series of the present experiment methylene blue was present, causing the potentials at the cell surface to pass into equilibrium with the dye system. But as the dye was progressively reduced the potential developed at the cell surfaces—not necessarily in the environment as a whole—traversed to a lower potential, thereby reducing trimethylamine oxide.

Since nitrate produced an inhibiting effect similarly to methylene blue, it is apparent that irreversible systems are able to develop equilibrium potentials comparable to reversible systems. The fact that fumarate did not inhibit the reduction of the oxide throws further light on the phenomenon. It is shown in a subsequent experiment that fumarate can be activated as a hydrogen acceptor in a manner comparable to trimethylamine oxide in the anaerobic growth of *reducing Achromobacter*. These results indicate that the failure of fumarate to inhibit the reduction of the oxide as did the nitrate was not because of the inability of the organism to activate this acceptor. If the potentials set up at cell surfaces pass into equilibrium with potentials of irreversible systems of nitrate, fumarate and trimethylamine oxide in the sense of reversible systems as discussed by Cannan, Cohen, and Clark (1926) this phenomenon could be explained by the relative position of these compounds in the potential series. In other words, fumarate was not inhibitive because it is lower than trimethylamine oxide in the potential series,

while it is possible that nitrate is higher than the oxide in the series and was therefore an effective inhibitor. It must be understood, however, that while these results are in support of a chemical potential theory they are not conclusive. Meldrum (1934) pointed out that certain systems reduced methylene blue but not quinone, and suggested that it is not a case of chemical potential but rather
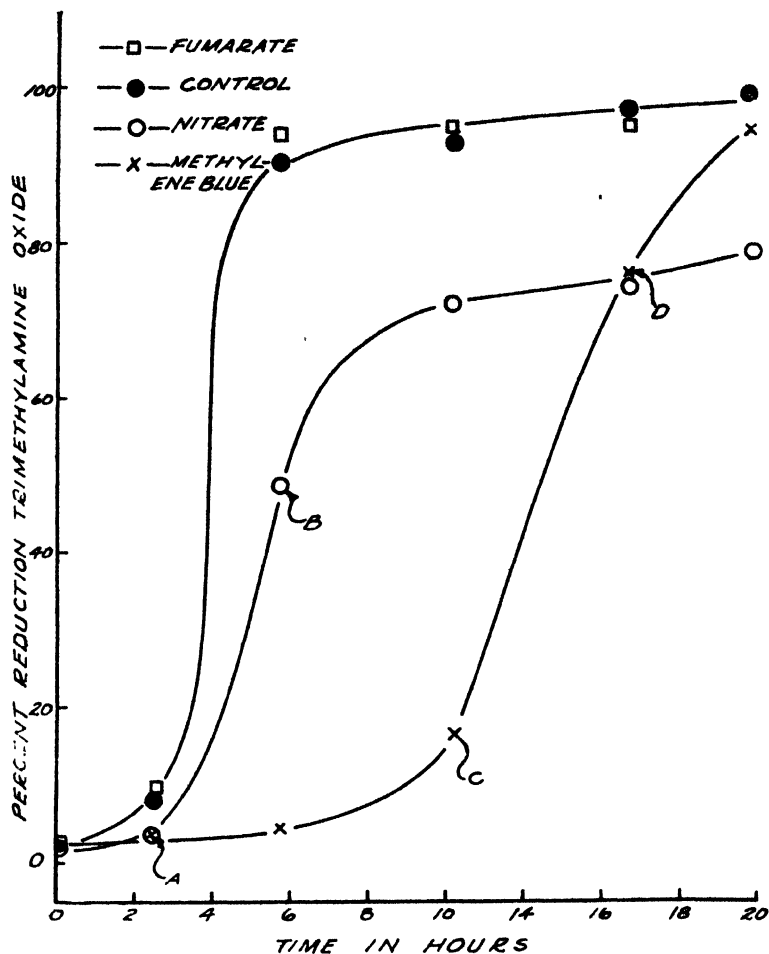
FIGURE 3. Influence of hydrogen acceptors on reduction of trimethylamine oxide during the growth of *reducing Achromobacter*.

of mechanism. A further study of this phase, including the determination of the apparent reduction potential óf trimethylamine oxide, should throw some additional light on the problem.

To support the conclusions derived previously, it was necessary to determine the ability of fumarate to function as a hydrogen acceptor in the anaerobic growth of *reducing Achromobacter*.

The principle of the method was similar to that used in the previous paper, based on the ability of hydrogen acceptors to support growth anaerobically. The

same technique was employed adding fumaric acid in the same molecular concentration as trimethylamine oxide. The experiment was adequately controlled employing fumarate-free media. As a further control, trimethylamine oxide and oxygen were also employed as hydrogen acceptors. The counts were made from a uniform suspension of *reducing Achromobacter*.
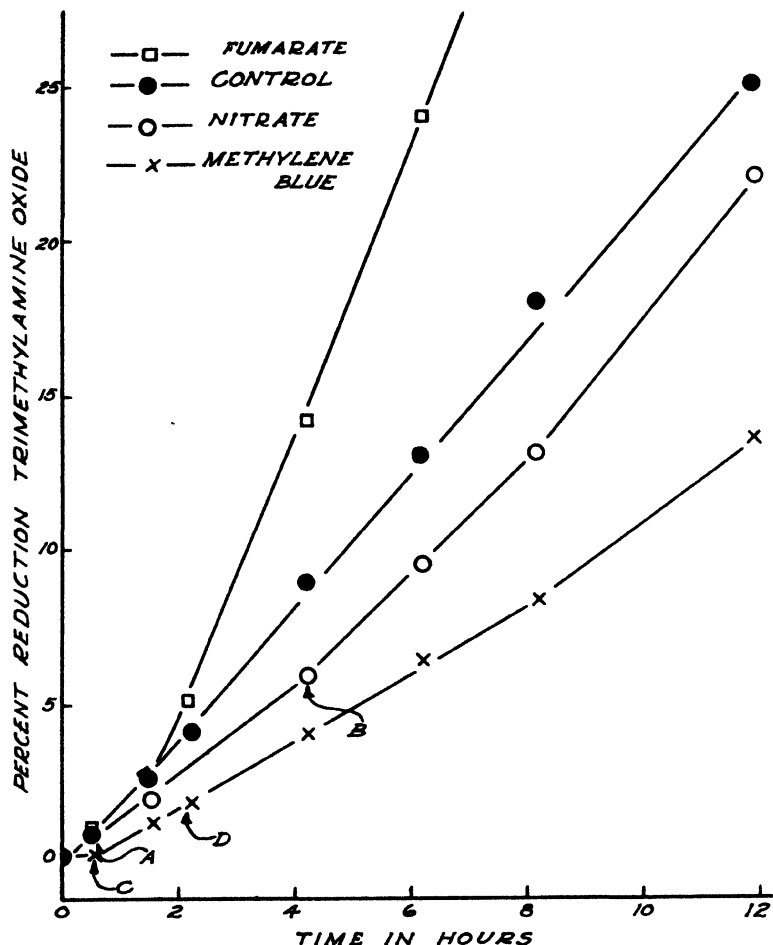
FIGURE 4. Influence of hydrogen acceptors on reduction of trimethylamine oxide in the presence of cell suspensions of *reducing Achromobacter*.

The results are recorded in table II. Since these counts check within the error of the method it is possible to conclude that fumarate functions as a hydrogen acceptor comparable to trimethylamine oxide and oxygen.

TABLE II. A comparison of fumarate, trimethylamine oxide, and oxygen as to their utilization as hydrogen acceptors in the growth of *reducing Achromobacter*.

Acceptor	Fumarate		Trimethylamine oxide		Oxygen	
Bacteria (no. per ml. × 10⁹).	18.0	16.6	15.5	16.0	18.8	16.0
	16.5	14.5	15.8	14.0	15.8	16.5

QUANTITATIVE RELATIONSHIP

It has been suggested earlier in the paper that trimethylamine oxide in solution at the cell surface becomes activated in the sense of Quastel (1926); in other words, if there is a suitable hydrogen donator present such as lactic acid, it becomes activated by the dehydrogenases; the hydrogen from the donator is transferred to and accepted by the oxygen from the oxide. Thus the first step in the reaction is as follows:—

$$CH_3CHOHCOOH + (CH_3)_3NO \rightarrow CH_3COCOOH + (CH_3)_3N + H_2O.$$
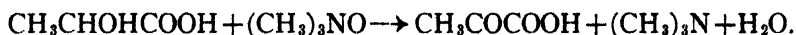
If this reaction does take place, pyruvic acid should be present in the reaction solution. In several preliminary experiments, tests were carried out for pyruvic acid, employing the method of Jowitt and Quastel (1937) based on the formation of its 2-4 dinitrophenylhydrazone; a positive test was never obtained. The reason for these negative tests is obvious on examining figure 2; it will be observed that pyruvate was oxidized at a greater rate than lactate as measured by the reduction of trimethylamine oxide. As lactic acid was oxidized, therefore, the pyruvate formed was also oxidized at a rate comparable to its production. The difficulty in finding pyruvate as an end product in reaction solution has been a common observation among other workers (Anderson 1938). Preliminary tests on the relation of oxide to lactate revealed the production of carbon dioxide. From these findings the reaction was considered to be:

$$CH_3COCOOH + (CH_3)_3NO + H_2O \rightarrow CH_3COOH + (CH_3)_3N + CO_2 + H_2O.$$
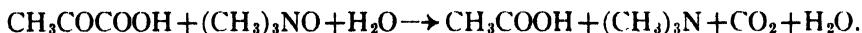
In the sense of Wieland (Krebs 1937) it is essential for water to take part in this reaction; in other words, a hypothetical hydrate is formed. The oxygen from the trimethylamine oxide is not accepted by the ketonic group from the pyruvate, but it accepts the hydrogen from the hydrate to form water. The validity of the participation of water is substantiated when the oxidation of pyruvate takes place in the presence of fumarate which in turn accepts the hydrogen to form succinate (Krebs 1937).

Employing the methylene blue technique, it has been proved that *reducing Achromobacter* cannot utilize acetate as a hydrogen donator. Assuming, therefore, that carbon dioxide is not activated as hydrogen acceptor the equation may be written:

$$CH_3CHOHCOOH + 2(CH_3)_3NO + H_2O \rightarrow CH_3COOH + 2(CH_3)_3N + CO_2 + 2H_2O.$$
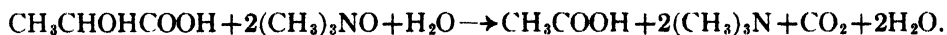
To prove the validity of the above equation quantitative determinations were made on the reactants and two end products after the completion of the reaction. It was necessary that the reaction be carried out in an oxygen-free environment; a reaction tube was designed, therefore, based on the inverted U-tube of Quastel and Whetham (1924). A sketch of the tube is shown in figure 5. The two chambers are connected so that after the replacement of the oxygen with nitrogen the contents of each chamber can be mixed. This apparatus has been employed for the methylene blue technique as described earlier.

It was necessary to control this experiment thoroughly, since it has been shown that there is a slight reduction of the oxide in the absence of the donator. Wilson (1938) attributed this phenomenon to a slight endogenous metabolism.

Three reaction tubes, therefore, were employed, one containing all the reactants, and the remaining two acting as controls. The first tube was prepared by adding a bacterial suspension of *reducing Achromobacter* in arm A (figure 5); in the other arm was placed a solution of trimethylamine oxide and a quantity of lactic acid in excess of that required to reduce the oxide completely. These solutions were buffered at a hydrogen-ion concentration of pH 6.9. The final concentration of bacteria was of the order of 4×10^9 cells per ml. The controls were prepared in the same manner; the first control contained lactic acid but no trimethylamine oxide; the second control contained trimethylamine oxide but no lactic acid. The chambers were evacuated and filled with oxygen-free nitrogen. After the oxygen had been completely removed the bacterial suspension in chamber A was placed in chamber B with the reactants. The reaction solutions were analyzed after 48 hours' incubation at 25°C.

Lactic acid was determined by the method of Friedemann and Graeser (1933). The Beatty and Gibbons (1937) modification of the Parnas-Mozolowski apparatus
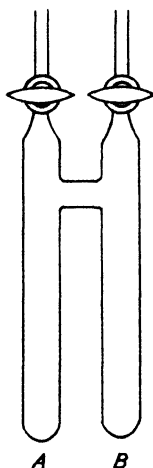
FIGURE 5. Sectional view of the reaction tube.

was employed for the trimethylamine determinations. Trimethylamine oxide was determined by Lintzel's (1934) method and carbon dioxide determinations were made in the Van Slyke volumetric apparatus. The average deviation between duplicate determinations has been recorded; it will be observed that these measurements are within 3 per cent.

The results of the analyses are recorded in table III. Since the trimethylamine oxide was completely reduced, the value 124 mg. per cent was placed in the completed equation and the theoretical values for the remaining reactant and end products calculated.

In the following, the theoretical values are compared with the values found by analysis:

	Lactic acid	Carbon dioxide	Trimethylamine
Theory................	74.5 mg. %	36.4 mg. %	98.0 mg. %
Found................	78.0 mg. %	34.2 mg. %	94.0 mg. %
(per cent of theory,	104.5	94.0	96.0)

These results confirm the theoretical equation within the limits of the methods. Since the equation held, the following general equation will express the reduction of trimethylamine oxide by *reducing Achromobacter* in the presence of an oxidizable substrate:—

$$AH_2 + (CH_3)_3NO \longrightarrow A + (CH_3)_3N + H_2O.$$
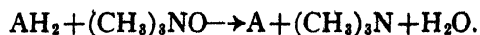
AH_2 is the oxidizable subtrate or hydrogen donator, oxidized in the presence of trimethylamine oxide to form the oxidized substrate A plus trimethylamine and water.

TABLE III.   Reactants and end-products in the oxidation-reduction reaction between lactic acid and trimethylamine oxide in the presence of *reducing Achromobacter*.

$(CH_3)_3N=O$ (mg. % in lactic acid free control)	$(CH_3)_3N=O$ (mg. % due to lactic acid)	$(CH_3)_3N=O$ (mg. % reduction due to lactic acid)
124.0 ± 2.0	-2.5 ± 2.5	124.0
Lactic acid (mg. % in oxide free control)	Lactic acid (mg. % in presence of oxide)	Lactic acid (mg. % reduction due to oxide)
348.5 ± 2.5	270.5 ± 3.5	78.0
CO_2 (mg. % in presence of lactic acid +oxide)	CO_2 (mg. % in oxide free control)	CO_2 (mg. % due to presence of oxide)
36.6 ± 0.0	2.4 ± 0.4	$34\ 2$
$(CH_3)_3N$ (mg. % in presence of lactic acid +oxide)	$(CH_3)_3N$ (mg. % in lactic acid free control)	$(CH_3)_3N$ (mg. % due to presence of lactic acid)
107.4 ± 1.3	13.4 ± 0.1	94.0

It should be recalled that of the five reactants and end products comprising the derived equation only four were determined experimentally; the fifth, acetic acid, has only mathematical confirmation. Because of the small quantities of the solutions available for analysis accurate determinations for acetic acid were not achieved.

GENERAL DISCUSSION

A comparative study of the dehydrogenase activity between *reducing* and *non-reducing Achromobacter* employing methylene blue and trimethylamine oxide as hydrogen acceptors has shown a further relationship between their respiratory

mechanisms. It is apparent, as formerly suggested, that the difference between the above groups is one of degree rather than kind; in other words, *non-reducing Achromobacter* have lost their ability to activate trimethylamine oxide as a hydrogen acceptor, probably as a result of the stimulus of the environment in the sense of Knight (1936).

The trimethylamine oxide *sparing effect* as observed by Watson (1939) on aerating fish muscle press juice has been demonstrated by the addition of small concentrations of hydrogen acceptors such as methylene blue, nitrate and fumarate; the latter failed to inhibit the reduction of the oxide. Since fumarate, however, was readily utilized in the anaerobic growth of *reducing Achromobacter*, there was evidence of a preferential activation of hydrogen acceptors, either as a function of their place in the potential series or as a function of mechanism. If the influence of these hydrogen acceptors is a function of their apparent reduction potential it is evident that trimethylamine oxide is slightly higher in the series than fumarate and lower than nitrate. The observation of Watson (1939) points to this latter relationship in that certain *Flavobacter* reduced nitrates readily but failed to reduce trimethylamine oxide. It has been recorded by Zobell (1932) that *Thiobacillus denitrificans* loses much of its reducing ability when cultivated under aerobic conditions; this phenomenon is apparently related to the oxide *sparing effect* and the adaptation hypothesis as presented in the previous paper.

The quantitative study of the coupled oxidation-reduction reaction involving trimethylamine oxide as hydrogen acceptor and lactic acid as the donator gave strong evidence for the following general equation:—

$$AH_2 + (CH_3)_3NO \longrightarrow A + (CH_3)_3N + H_2O.$$
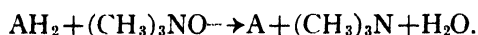
where AH_2 is the oxidizable substrate or hydrogen donator and A the oxidized substrate. Variations in dehydrogenase activity between the strain of *reducing Achromobacter* employed in the present experiment and other strains may produce discrepancies in the reaction between lactic acid and trimethylamine oxide as recorded in this paper. Regardless of possible differences in dehydrogenations, however, it is obvious that the principle of the reduction of trimethylamine oxide will hold according to the general equation.

Since Sharp (1934, 1935) has shown an accumulation of 0.40 to 0.45 per cent lactic acid in fish muscle and Beatty and Collins (unpub.) have shown lactic acid to disappear concomitantly with the production of trimethylamine in cod muscle press juice, the significance of the results as recorded in this paper becomes obvious. We have seen that two molecules of trimethylamine oxide were reduced for each molecule of lactic acid oxidized. Thus, considering the quantity of carbohydrates and their derivatives present in fish muscle it is evident that the bacteria present can reduce the total concentration of trimethylamine oxide employing only these substances as hydrogen donators. It is possible to infer, therefore, that trimethylamine produced in cod muscle press juice is a result of energy yielding reactions between trimethylamine oxide and appropriate hydrogen donators, especially carbohydrates and their derivatives, during the anaerobic respiration of *reducing Achromobacter*. These conclusions confirm the conception of fish spoilage in the sense of Beatty and Collins (unpub.).

SUMMARY

Trimethylamine oxide is reduced as a linear function of time by cell suspensions of *reducing Achromobacter* in the presence of a number of hydrogen donators, namely, glucose, glycogen, lactate, and pyruvate.

Suspensions of *non-reducing Achromobacter* are unable to activate trimethylamine oxide as hydrogen acceptor in the presence of the above donators.

Reducing and *non-reducing Achromobacter* show the same dehydrogenases as tested by the methylene blue technique in the presence of a number of different hydrogen donators. A further relationship between the groups is discussed.

The addition of small concentrations of hydrogen acceptors such as methylene blue and nitrate inhibits the reduction of trimethylamine oxide. Since fumarate is not inhibitive, but, like trimethylamine oxide, supports the anaerobic growth of *reducing Achromobacter*, there is evidence of a preferential activation of hydrogen acceptors.

A coupled oxidation-reduction reaction between lactic acid and trimethylamine oxide is tested quantitatively; a general equation involving the reduction of trimethylamine oxide in the presence of a hydrogen donator and *reducing Achromobacter* is derived from the results.

The results as applied to a conception of fish spoilage are discussed.

ACKNOWLEDGEMENT

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ULTRAVIOLET LIGHT AS A STERILIZING AGENT FOR BRINES OR PICKLES

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In a previous Progress Report (No. 38, p. 3, 1938) it was suggested that treatment with ultraviolet light (UVL irradiation) might prove a convenient method for partially sterilizing brines or pickles used in the curing of fish since, as has already been suggested (Report No. 37, p. 7, 1938), such liquids can be a serious source of contamination to fillets immersed in them.

To test the efficacy of UVL in this connection, a specially constructed sterilizing tank was placed, as shown in the accompanying figure, inside the irradiating cabinet described in Progress Report No. 38. A similar covered tank (not shown) containing no stirrer was placed beside the sterilizing tank in the cabinet, to contain untreated brines used as controls; during an experiment both the control and treated brines would be under as nearly identical conditions as possible. These tanks are 6 inches wide, 25 inches long and 9½ inches deep, lined with bright tin rounded at the bottom. The sterilizing tank has an UV-lamp running longitudinally through it about one inch above the bottom, and a basket made of ½-inch mesh galvanized wire screen for containing fillets revolves on its longitudinal axis above the lamp. This basket revolves at about 20 r.p.m. and whether empty or containing fillets, agitates the brine exceedingly well due to the rounded bottom of the tank.

The revolving basket was used since it has the advantage that fillets would constantly be washed by the almost sterile brine. The brine itself is rapidly circulated so that the bacteria must frequently be exposed to the higher intensities of UVL near the lamp and, if the rays penetrate sufficiently, the surfaces of the fillets would themselves be exposed evenly to the emanations. It must be emphasized in these experiments that two factors, namely UVL and the washing action of the brine, would probably each play a role in improving fish fillets treated in such a bath.

The general experimental procedure was as follows: Eight-gallon lots of 2, 5 and 15% (by weight) of sodium chloride brine were prepared, the resulting solutions being contaminated to various degrees by immersing in them slightly stale fish flesh which was later removed. After thoroughly mixing, the number of viable organisms (those capable of growth) was determined and approximately 3½ gallons were dispensed
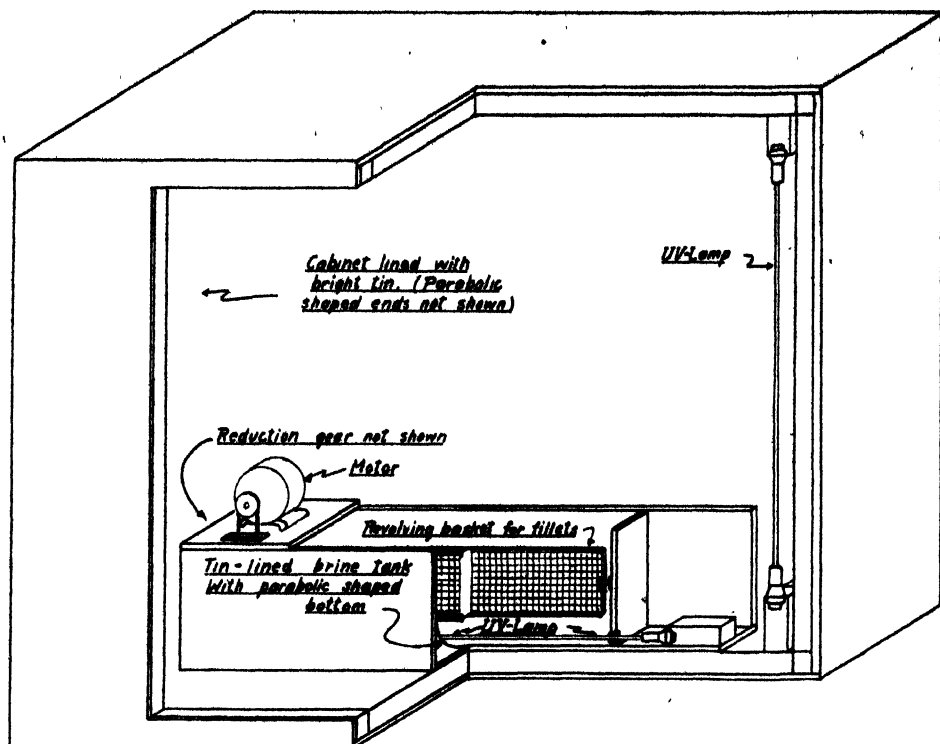
into each of the two experimental tanks. The brine in the control tank was left undisturbed, while that in the other tank was irradiated for given lengths of time. Bacterial counts were made on these brines at stated intervals.

The results of three typical experiments show that the viable bacteria present in contaminated brines are practically all rendered incapable of further growth after the UVL treatment:

The 2% brine originally contaminated with 800,000 viable organisms per cc. (0.06 cubic inch) still contained practically as many bacteria after 3½ hours; but upon exposure to UVL for the same length of time, only six viable bacteria per cc. remained.

The bacterial content of the 5% untreated brine originally contaminated with 1,750,000 organisms per cc. was practically unchanged after 3 hours, whereas with irradiation the 1,750,000 had been reduced to forty-four in 2 hours and was further reduced to six after 3 hours.

The bacterial content of the 15% untreated brine containing about 9000 bacteria per cc. showed no significant change in 2 hours; exposure to UVL reduced the original contamination to four hundred in 1 hour and to twenty-eight in 2 hours.

The temperatures of the treated brines varied between about 45° and 65° F., the temperature of the brine in contact with the U.V. lamp being naturally somewhat higher than that of the control brine.

Treatment with UVL has one distinct advantage over heating a brine in order to sterilize it, since in the first-named treatment the bacteria are being killed during the actual brining of the fillets, as experiments not reported here have shown. Whether or not the keeping quality of fresh or smoked fillets is materailly improved by the prior brining in brines which are almost sterile bacteriologically, rather than in untreated contaminated brines, is now being investigated. Factors such as initial contamination of the fish and its freshness, salt concentration of the brine, etc., in connection with chemical treatments, will almost certainly be important in determining the effectiveness of UV irradiation, as is pointed out in another report in this issue dealing with chemical treatments. It is intended to describe the results of treating fish in contaminated and irradiated brines later.

THE ROLE OF PRESERVATIVES IN ENHANCING THE KEEPING QUALITY OF FRESH FILLETS.

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The addition of various preservatives to fish or fish products in attempts to improve their keeping qualities has long been advocated. In 1870, Norwegian exporters succeeded in preventing to a great extent the deterioration of salted herring shipped to England by adding boric (boracic) acid to the pickle. There is no doubt that the use of chemical preservatives in, or upon, fish or fish products has been markedly influenced by the pure food laws enforced in different countries. These laws vary greatly. Thus in Germany and the Scandinavian countries considerable freedom is permitted with respect to the addition of preservatives to such foods, and consequently much work has been done in these countries to determine the suitability and effectiveness of various chemicals which are apparently harmless to the human organism. On the other hand in Great Britain and Canada the number of permitted preservatives used in connection with edible fish products is strictly limited, and less research has been carried out along these lines. In the United States there appears to be some freedom as regards the use of preservatives in fish. In general, it appears that most countries permit the export of fish products with added preservatives, provided that the nature and quantity of the preservative is declared upon the package.

Only a very brief and general survey of the work which has been done on the use of preservatives in connection with fish and products derived therefrom can be presented here. A search of the literature has shown that attempts have been made to improve the keeping quality of round (dressed) fish by immersing them, soon after gutting, for relatively short periods in solutions containing sodium hypochlorite (active chlorine), formaldehyde (formalin), boric (boracic) acid, saltpeter (potassium nitrate), or strong solutions of common salt, prior to packing them in ice. Other work has been concerned with the use of sterile or sterilizing (antiseptic) ices containing hypochlorites, ozone, or benzoic acid; or with the storage of fish in atmospheres containing large amounts of carbon dioxide. With few exceptions these experiments with round fish have met with only slight success or with failure, undoubtedly because of the difficulty of reaching and killing the bacteria which gain access to the fish muscle through the rough cut surfaces at the time of dressing and scraping.

The use of preservatives during the preparation of fillets, or in various other fish products, naturally offers far greater possibilities because of the more intimate contact afforded between the chemical and putrefactive agent. Thus both boric and benzoic acids (or their soluble sodium salts) have been employed for many years to control the reddening or bacterial decomposition of split salted fish. In Germany the keeping quality of such products as "marinated fish", shell-fish, salmon, spiced herring, anchovies, fish roes, caviar, crabs, etc. has been

greatly improved by the addition of benzoic acid and related chemical compounds, boric acid, etc. Other substances which have been used, or the use of which has been suggested in this connection, include para-hydroxy benzoic acid and its methyl, ethyl and propyl esters, hexa-methylenetetramine, chloroform, ether, sulphur dioxide, carbon disulph-ide, sodium bisulphite, alum, thymol, sodium fluoride, salicylic acid, alcohol, lactic acid, tartaric acid, citric acid, formic acid, formaldehyde, etc. More recently, hydrochloric acid, hydrogen peroxide and borates have been used in attempts to increase the keeping quality of fresh fillets in Norway, Germany and in Eastern Canada, but the results have not always been conclusive.

Since in the future the demand for wrapped and packaged un-frozen and frozen fillets seems likely to increase, the writers have been investigating various methods of enhancing the keeping quality of such products. Quick-frozen fillets are rapidly gaining popularity, but the interval between dispensing them from the last properly refrigerated holding chamber, and the ultimate utilization of the fillets, frequently is of such duration that a treatment of the fillets in some preservative prior to freezing is worthy of consideration.

In a recent Progress Report (No. 37, p. 7, 1938) the writers noted that a brief immersion in brines containing benzoic acid slightly improved the keeping qualities of fillets which were subsequently smoked, but other organic acids tried had less effect. Another article in the present issue reports progress being made in the investigation of the use of ultraviolet light to reduce the numbers of bacteria coming in contact with fillets. The results of further experiments on the use of preservatives in connection with fillets are given below and in the accompanying table. It is intended to publish the results more fully in due course.

The present experiments were carried out using fillets prepared from recently-caught "flounders" or from frozen halibut which had been stored from 4 to 6 months at -12° F. The standard procedure was to immerse small experimental fillets for one hour in a 2% common salt brine at a temperature of about 77° F., to which the various preservatives had been added (see table). The experiments have, however, been varied somewhat in order to study the influence of such factors as salt concentration, length of immersion and preservative concentration on the keeping quality. As far as we have observed, brines having a concen-tration of up to 2% common salt have no appreciable effect in reducing the bacterial contamination of fillets dipped therein. Stronger brines (5% salt and over) commence to have a noticeable effect on some bacteria, but are not practicable for the preparation of certain "fresh" fish products.

After treatment, the fillets were stored at 35° F. in sterilized covered glass beakers and examined as soon as the control fish (those immersed in the brine without preservative) were definitely stale or putrid. Four or more fillets were used for each determination, the criteria of keeping quality being bacterial count, odour of the uncooked and cooked flesh, and taste. For the purposes of experiments with preservatives intended to inhibit bacterial multiplication, determinations of the numbers of bacteria in the fish appeared to give the best index of the relative activity of the chemical employed. Certain chemical tests suitable under some circumstances as criteria of freshness were for various reasons unsatisfactory in testing these products.

In the accompanying table giving the results of preliminary observations, the figures in the third and fourth columns are the factors by which the numbers of surviving bacteria found in the samples after treatment would have to be multiplied to indicate the original number of bacteria in the samples; the greater the factor in these columns, the greater the bactericidal and presumptive preservation action of the chemical.

Inhibition of bacterial growth caused by treating fish fillets with different preservatives.

Preservative added	Percent preservative in brine	Factor of decrease in bacterial count in fillets from	
		fresh flounder	frozen halibut
Sodium benzoate	0.1	9.7	0.7
	0.5	429	—
	1.0	7520	—-
Benzoic acid	0.1	33, 30, 5.6	1.1
Potassium nitrite	0.02	—	6.1
	0.05	. ---	48.2
	0.1	167	1450
Hydrogen peroxide	0.1	2.3	—
Chloroform	0.7	304	—
Boric acid	0.1	9.8	—
Hydrochloric acid	0.07	53.4	—
Sulphur dioxide	0.1	104	—
Para-hydroxy benzoic acid ethyl ester	0.09	3	1.4

The results obtained are of some importance, particularly with regard to our observation that nitrite, in quite low concentrations, very strongly inhibits the rate of spoilage of fillets. In this connection it is interesting to observe that in Canada the regulations under the Food and Drugs Act (Ottawa, 1938) state that refined sodium nitrite in a concentration of 0.02% can be used in cured meats and fish. Hydrogen peroxide, hydrochloric acid and boric acid in the concentration employed were not particularly effective. The use of hydrochloric acid and sulphur dioxide, under the experimental conditions, resulted in a product of rather unpleasant appearance, the surface of the fillet being considerably roughened and whitish. Sulphur dioxide produced an extremely unpleasant odour and flavour in the treated fillet, probably because it reacted with the oils present. Chloroform proved to be very active, and it has the advantage that it later evaporates on storing the fillets. While both boric acid and para-hydroxy benzoic acid ethyl ester prevented deterioration considerably, neither was as effective as sodium benzoate or benzoic acid. Benzoic acid was, as might be predicted, more active than sodium benzoate when used in about the same concentration; however, the free acid has the disadvantage that it produces a slight roughening of the surfaces of treated fillets. Since the differences were not very marked, and since benzoic acid is neutralized when it enters the fish muscle, it would appear that the sodium salt is almost as efficacious as the acid itself.

It will be seen that the inhibition in bacterial growth was not by any means always the same with a given preservative, particularly with the different kinds of fish. This is evidenced by comparing the results with sodium benzoate and benzoic acid as applied to the flounder and halibut fillets. The value 0.7 for the halibut fillets treated with benzoate indicates that the contamination after treatment was greater than before. There is no doubt that many factors may influence the activity of a preservative. In fillets cut from fresh fish the contamination is probably chiefly superficial as has previously been pointed out (Prog. Rep. No. 38, p. 3, 1938), and the chemical is more likely to prove effective than in old fillets in which the bacteria are probably more or less evenly distributed through the tissues. Factors such as the rate of diffusion of the chemicals into the fillets, the salt concentration of the brine, etc. may also influence the effectiveness of a given chemical treatment.

Before concluding it must be noted that the results of bacteriological tests have in most cases been freely borne out by organoleptic tests in the experiments cited, except in cases where the chemical actually caused an undesirable flavour (particularly with sulphur dioxide.) It is hoped that further experiments will throw additional light upon the value of certain of the more effective and possibly acceptable preservatives in improving the keeping quality of both fresh and smoked fillets. So far, these experiments have shown that many of the chemical preservatives studied have been more efficient in reducing the number of viable bacteria on treated fresh fillets than has ultraviolet light.

505

RESPIRATION CURVE FOR McINTOSH APPLES[1]

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A study of respiration by measuring CO_2 output has for some time proven to be a valuable means of diagnosing the storage behaviour of apples. The form of the resulting curve obtained by plotting CO_2 output rates against time, as well as a study of actual rates, and the transition of the curve on change of environment, have contributed valuable data for respiration studies.

The characteristic trend of CO_2 output rates of fruit commences with a rising line shortly after fruit set until a peak is reached about mid-season; then a falling line is evident until harvest, after which another similar rise and fall ensues. Thus the usual complete curve is one consisting of a double hump. The second hump or climacteric is of extreme interest when making a study of storage behaviour.

During the summer of 1936 arrangements were made to study the respiratory rates of McIntosh apples with the primary object in view of determining how closely the senescent respiratory curve conformed to the characteristic trend determined for other fruits. For this purpose a healthy vigorous tree in an apple orchard at Iroquois was selected. The entire crop of one limb on this tree was harvested when the fruit was considered to be at the proper harvest stage for storage. Some of these apples were selected for immediate study and the remainder were placed in storage. Samples of these latter were removed from storage at intervals throughout their storage life in order to establish the trend of respiration under storage conditions.

The temperature at which all CO_2 output records were made was 55° F. This temperature was maintained by immersing the single-apple respiration chambers in a water bath. An air stream free of CO_2 was passed over each apple. After leaving the chamber, the air stream was bubbled through Pettenkofer tubes containing a standardized barium hydroxide solution. These tubes were emptied and titrated daily, and from this the mean hourly rate of CO_2 output of each apple was calculated. Daily records were also made on external observations of the condition of the fruit.

The results of the first year's work indicated that the respiration rates assumed a downward trend from harvest time until the end of storage life. This was contrary to the anticipated results. The expected senescent hump was missing entirely in all trials.

There were two possible explanations for this phenomenon, either McIntosh did not pass through a senescent hump phase or, if it did, this hump occurred previous to harvest.

For the second year's work the identical source of the fruit as for the first year was used. Instead of waiting until harvest, however, the first sample was picked on August 25. Successive samples were picked on August 29, September 22, and October 5. The last sample was approximately the same maturity as was all the fruit used for the first year's work. All apples were placed in the respiration chambers within 24 hours of picking.

The resulting respiratory curves are shown in Figure 1. It will be seen that the curve for the earliest picked sample (August 25) commences with a slightly ascending line which continues for 8 or 9 days. At this point some of the fruit shows an extremely sharp rise and fall after which a more gradual falling trend is assumed. This latter trend resumes the approximate air line level it assumed previous to the sharp rise. Some of the fruit failed to produce this rise. This absence or reduction of the climacteric in extremely immature fruit was noticed by Kidd and West (2) in Comice pears, but they failed to find it in apples.

The second pick fruit (September 9) assumes an entirely different trend. These apples all produce descending rates which continue for about 10 days after which time ascending rates occur. After a maximal point is reached the rates show a gradual decrease. This descending phase is not nearly as steep as the previous increasing phase. It will be noted also that the hump does not reach as high a maximal point as in the apples of the first pick, but the subsequent trends after the peak are practically the same.

The curve for third pick fruit is not radically different from that of the second pick. There are certain differences, however, in that the third pick starts with a level phase rather than a falling phase, after which the rates increase to a maximal point which is followed by a gradual falling phase. This latter phase is higher than in the first and second pick.

In the fourth and last pick there are produced two types of curves. In one type an initial rising phase occurs, while in the other there is a falling phase which continues throughout. The apples with the falling line approximate the circumstances of the first year's results. Those showing an initial rising line are probably slightly less mature.

Evidently the same curve does not occur on the tree as is represented after the apples are detached. If this were so the initial rates of successive pickings would approximate the curve of the previous picks at the same time. The chief difference to be noted in this regard is that the senescent hump is hastened by detachment. If we wish to approximate the respiratory condition on the three therefore we can only do so at the initial points after detachment from the tree. If these initial points are plotted, the resulting curve would appear as indicated in Figure 2.

Further examination of Figure 1 shows considerable divergence in the minimal values previous to the climacteric rise in the first three picks. What is of interest is that the difference between this minimal rise and the climacteric peak is quite close in all three cases, viz., 11.5, 11.0 and 9.0 mgms. of CO_2 per kgm. hour. The difference that does exist, however, is a tendency for the increasing slope of the climacteric to decrease with maturity.

FIGURE 1: THE RESPIRATORY ACTIVITY OF McINTOSH APPLES REMOVED FROM THE TREE AT DIFFERENT DATES.
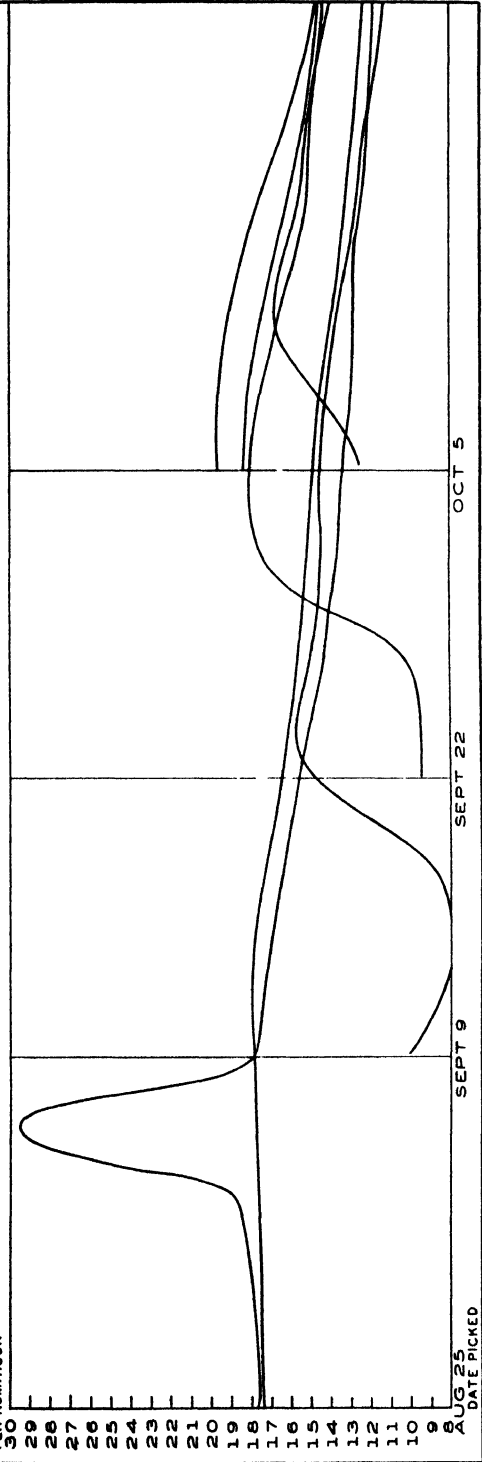
FIGURE 2: TREND OF RESPIRATORY ACTIVITY OF McINTOSH APPLES PRIOR TO AND AFTER HARVEST CALCULATED ON THE BASIS OF INITIAL RATES AFTER PICKING.
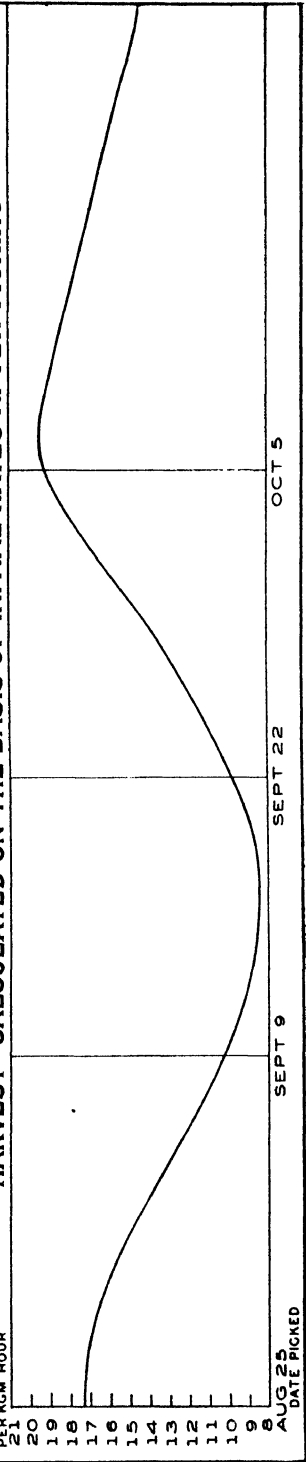
If the generation of an auto-stimulant theory of Kidd and West (1) is assumed, it would then appear that the total effect is very nearly equal in all picks. As maturity progresses however, there appears to develop a state of metabolism which tends to oppose the effect of the auto-stimulant.

DISCUSSION

The outstanding conclusion to be made is that a senescent hump or climacteric does occur in McIntosh apples. From this year's work it appears to occur about the end of September. This point coincides with the time when starch is rapidly disappearing and the ground colour is becoming yellow. -

On considering the storage behaviour of this variety it would seem that the climacteric phase must be passed on the tree if maximum storage quality is going to be obtained. In other experiments designed to establish a co-relation between maturity and core flush development in storage it would appear that an appreciable reduction in core flush would result from allowing McIntosh to pass the climacteric phase on the tree.

In an experiment (4) embracing the study of methyl bromide as a fumigant for apples, it was found that if fumigation were delayed for several weeks after harvest, less injury resulted as compared to apples fumigated immediately after harvest. Hence it would appear that if the fruit is allowed to pass the climacteric and settle down to a stable state of metabolism, less injury ensues from the methyl bromide fumigant.

It was found in gas storage experiments that McIntosh exposed to a 7% CO_2 and 14% O_2 mixture at 39° F. before reaching the climacteric suffered from CO_2 injury. No injury was found in the same treatment with mature McIntosh apples which would probably be at the same stage as pick 4, at time of harvest. These facts suggest that McIntosh apples have a different degree of susceptibility previous to the climacteric. Kidd and West (3) have shown that a temporary increase in CO_2 output is produced in gas storage in the pre-climacteric phase in certain varieties. This could not influence the concentration in the gas chamber of the previous experiment since the work was conducted in continuous flow gas chambers with ample air flow to take care of this increase.

It can be seen that if the occurrence of the climacteric could readily be determined while the fruit is still on the tree it would be of immense value to the fruit grower in selecting fruit for storage.

An attempt is being made to co-relate ethylene production with the climacteric rise. If the method proves satisfactory, a more suitable means of determining the climacteric may result. From this information, the grower could be more reliably informed as to the proper harvest time for best storage results.

SUMMARY

Respiration studies on individual McIntosh apples at 55° F. revealed only a falling trend during the first year's work. Investigations during the second year's work revealed the fact that the senescent hump in respiration occurs at or just previous to the time of harvest. From experimental

evidence it appears that McIntosh store better and develop much higher quality if the senescent hump phase is passed on the tree. In other experiments it was also shown that if apples were allowed to pass the climacteric phase they were much less susceptible to CO_2 or methyl bromide injury.

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BROWN ROT OF PEACHES IN TRANSIT AND STORAGE

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BROWN ROT OF PEACHES IN TRANSIT AND STORAGE[1] [3]

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Brown rot, caused by *Sclerotinia fructicola* (Wint.) Rehm., is of such outstanding importance in practically every peach-growing district of the world, that it is almost trite to remark that the Ontario peach industry has endured heavy losses from the disease in the orchard, during transportation, on the market, and even in the hands of the consumer. Most of the investigations on brown rot in the past have been concerned mainly with control in the orchard, and rightly so, because failure there is of primary importance. The present studies, on the contrary, have been directed chiefly towards acquiring information concerning the effectiveness of control measures and the behaviour of the disease after the fruit is harvested, for, very often, while there may be little or no rot evident in the orchard, considerable may develop in the course of long distance transit, for example to the Canadian West, and even in the shorter hauls to nearby points.

REVIEW OF LITERATURE

The literature on brown rot and its control is so voluminous that no attempt will be made in the present instance to deal exhaustively with the subject, a detailed and comprehensive discussion of which may be found in the bulletin of Roberts and Dunegan (19). The consensus of opinion (7, 12, 18, 19, 20) is that effective orchard control can be achieved by careful sanitary measures and a series of summer sprays. Sanitation involves the removal and destruction of blighted twigs and mummied fruits. For the most part, the spray programmes are based on the schedule introduced by Scott (21), and comprise a pre-blossom spray of wettable sulphur, or lime-sulphur 1 : 40; a "shucks" spray of self-boiled lime-sulphur or wettable sulphur, with poison where necessary for the control of curculio, and a spray or dust, without poison, applied three or four weeks before harvest. However, to this programme there are a number of variants in both materials and timing (6, 7, 9, 12, 14, 16, 17, 18, 19, 25). According to Snapp, Alden *et al.*(17) spraying gave more effective control than dusting.

Several investigators (2, 4, 5, 10, 22) have expressed the opinion that rots developing in transit and in the market may be considerably reduced by spraying or dusting. Brooks and Cooley (3) state that, while brown rot develops at a lower temperature on peaches than on potato dextrose agar and at a lower temperature on ripe peaches than on green, its development is delayed longer the nearer the temperature approaches freezing. In a later paper, these authors (4) indicate that low temperature had a relatively greater inhibitory effect upon the development of rot during the incubation period than during later growth. For that reason there was

greater damage from rot when cooling was delayed, than when cooling was effected immediately after harvest, especially in inoculated peaches. They also found that both the temperature and rate of cooling in refrigerator cars varied greatly from place to place in the car, and from the top to the bottom layers of boxes.   To secure greater uniformity of temperature in iced cars, Brooks (2) suggested precooling, the use of car blowers, or the addition of salt or dry ice to the ice in the bunkers.   Hienton and Fawcett (15) advocate that salt be added to the ice, and that peaches be packed in the car in such a way, and in such containers, that the air may circulate freely about the fruit.

Brooks (2) found a positive correlation between shipping losses in Georgia peaches and the rainfall during the growing season in the case of brown rot, but not when *Rhizopus nigricans* was involved.   Anderson (1) and Fish (10) consider *R. nigricans* to be of greater importance than *S. fructicola* as a transit rot.   Moreover, *R. nigricans* is not amenable to control by spraying (1, 10) but seems to be dependent on picking, packing and shipping conditions rather than on factors operative in the orchard (2, 10).   *R. nigricans* is more readily checked by low temperatures than is *S. fructicola* (3) and can be reduced by sanitation in picking and packing and by sterilizing the equipment (10).

The conclusion of Dorsey and Potter (8) that brushing or "defuzzing" peaches would facilitate infection by allowing spores to make more direct contact with the surface of the fruit, was confirmed by the experimental work of Smith (26).   Smith (24) also demonstrated that dusting-sulphur applied before inoculation with fresh spores of *S. fructicola* was effective in controlling brown rot in both brushed and non-brushed peaches held at 45 to 55° F., but not at 65° F. or higher.

MATERIALS AND METHODS

Peaches of the varieties Rochester and Elberta were used throughout the experiments.   The orchards were divided into experimental plots according to the fungicide used.   The procedures followed during picking and packing operations and in the taking of observations in the years 1933 to 1937, were formulated as the result of some preliminary trials in 1932. Fruit from each plot was picked in the morning and immediately taken to the packing house to be sorted by visual inspection into three grades, (i) "green" in which the fruit was mature enough to ripen at room temperature, but had not yet changed appreciably in colour; (ii) "intermediate" in which the ground colour was beginning to take on the yellow shades; (iii) "ripe" in which the ground colour was definitely yellow and the fruit was beginning to soften but was not yet ripe enough to be eaten out of hand.   As the sorting proceeded, the peaches were wrapped in tissue paper and packed in 2-layer lugs, so that packing was completed on the day of harvest.   Throughout these operations the peaches were carefully handled, except in specified instances, in order to minimize bruising or puncturing. Care was also taken to avoid handling rotting fruits while collecting the experimental samples.   Peaches from each lot were tested for firmness by means of a pressure-tester, and in the first 2 years their ground and flesh colour were recorded after comparison with Ridgeway's colour chart.

Shipments, comprising one or two boxes of each lot, were sent in iced cars at a temperature of 45° to 50° F., to Winnipeg or to Saskatoon, distances of 1,000 and 1,500 miles respectively. In most cases, duplicates of these shipments were held for examination at St. Catharines. Each year also, a transatlantic consignment of Elberta peaches was shipped to England via port of Montreal.

Observations were taken at intervals of 3 or 4 days over a period of 2 to 3 weeks after picking in the case of lots examined at St. Catharines, and at similar intervals for a week or 10 days after arrival in the case of shipments. The peaches were handled in the wraps and not touched directly by hand during the various examinations, at each of which any fruits showing spots of rot were recorded and discarded, regardless of the extent of the rot. These methods were employed to avoid, as far as possible, both contamination during handling and undue spread of rot by contact during the intervals between examinations.

OBSERVATIONS

The graphs (Figures 1 to 5), which present the data obtained from the various lots or shipments, although apparently 3-dimensional in form, should really be considered as multiplanar. Each consists of two intersecting sets of parallel planes, arranged to facilitate the comparison of 2 sets of interacting conditions or factors. Dates of spraying and of precipitation during the growing seasons from 1933 to 1937 are given in Figure 6.
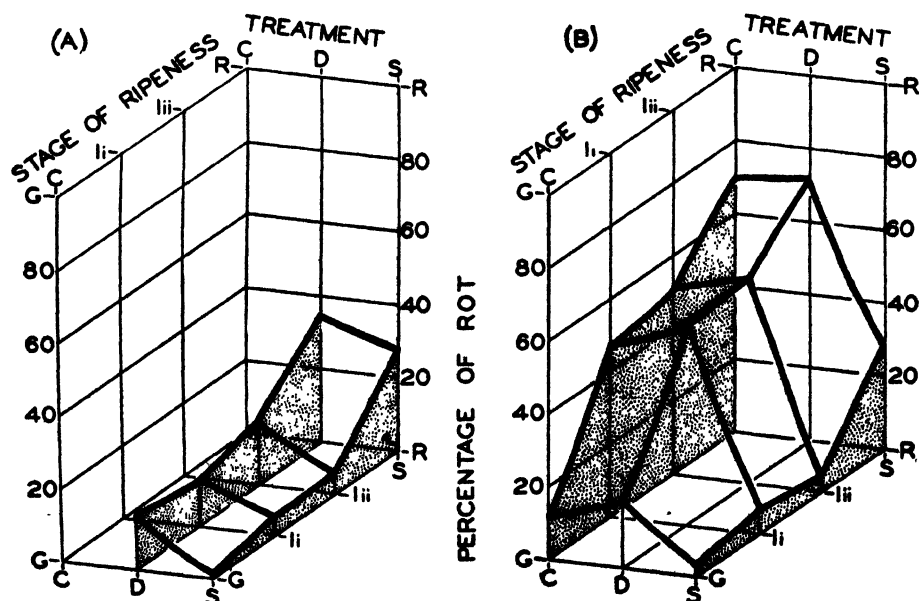
FIGURE 1. Incidence of rot, in relation to treatment and stage of ripeness at harvest, in 1933 in Rochester peaches: (A) examined at Winnipeg; (B) at St. Catharines. Stages of ripeness: G, greenest grade of pick; Ii, Iii, intermediate; R, ripest; all except Iii carefully handled. Treatment: C, check; D, dusted; S. sprayed with Koloform. See also Table 1.

Experiments in 1933

In the first pick of Rochester (Figure 1, A) the checks were omitted from the record as not comparable with the treated peaches, for not only were they taken from a different orchard but also an unsuccessful attempt was made to sort them during picking.   In the second pick (Figure 1, B), while the dusted peaches were from the same source as in pick 1, and the checks and sprayed peaches were from different orchards, the samples were more nearly alike, as indicated by the pressure tests.   That the percentage of rot in the sprayed peaches of the second pick on the fourth day at room temperature was about the same as that of similar fruit of the first pick on the second day, was largely due to the lower temperature during the holding period for the second pick (Table 1).   The failure of the dust in the second pick was undoubtedly due to the heavy rainfall immediately preceding harvest (Figure 6).   The rain evidently had little effect on the spray.

TABLE 1.—DATA RELEVANT TO PICKS OF ROCHESTER PEACHES IN 1933 (SEE ALSO FIGURE 1)

Reference in Fig. 1	Date of		Observations			Room temp., °F.*	Remarks
	Pick	Arrival	Date	Days after harvest	Days at room temp.		
A	Aug. 16	Aug. 21	Aug 23	7	2	72–80 (78)	Shipped to Winn. Aug. 16.
B	Aug. 18	Aug. 26	Aug. 30	12	4	62–72 (68)	Held at 33–36° F. till Aug. 26.

* Range and estimated mean (in brackets) of temperature during the period indicated in the previous column.

With the possible exception of the third pick of Elberta, held in the slightly damper atmosphere of the cellar (Figure 2, C; Table 2), dust and spray gave approximately the same degree of control and both showed considerable improvement over the checks.   It should also be noted that transportation to Saskatoon (Figure 2, A) in a properly iced car had little

TABLE 2.—DATA RELEVANT TO PICKS OF ELBERTA PEACHES IN 1933 (SEE FIGURE 2)

Reference in Fig. 2	Date of		Observations			Room temp., (°F.)	Remarks
	Pick	Arrival	Date	Days after			
				Picking	Arrival		
A	Sept. 8	Sept. 14	Sept. 21	13	7	57–80 (65)	All but liii precooled; shipped to Saskatoon Sept. 9.
B	Sept. 5	Sept. 9	Sept. 13	8	4	66–70 (68)	Shipped to Winnipeg Sept. 5.
C	Sept. 11	Sept. 11	Sept. 18	7	7	58–70 (65)	In cellar at St. Cathar-
D	Sept. 11	Sept. 11	Sept. 18	7	7	60–80 (70)	In laboratory ines.

or no ill effect or the fruit, since the amount of rot after a week at room temperature and almost 2 weeks after harvest compared favourably with that in the lots examined at St. Catharines (Figure 2, C, D) after a week
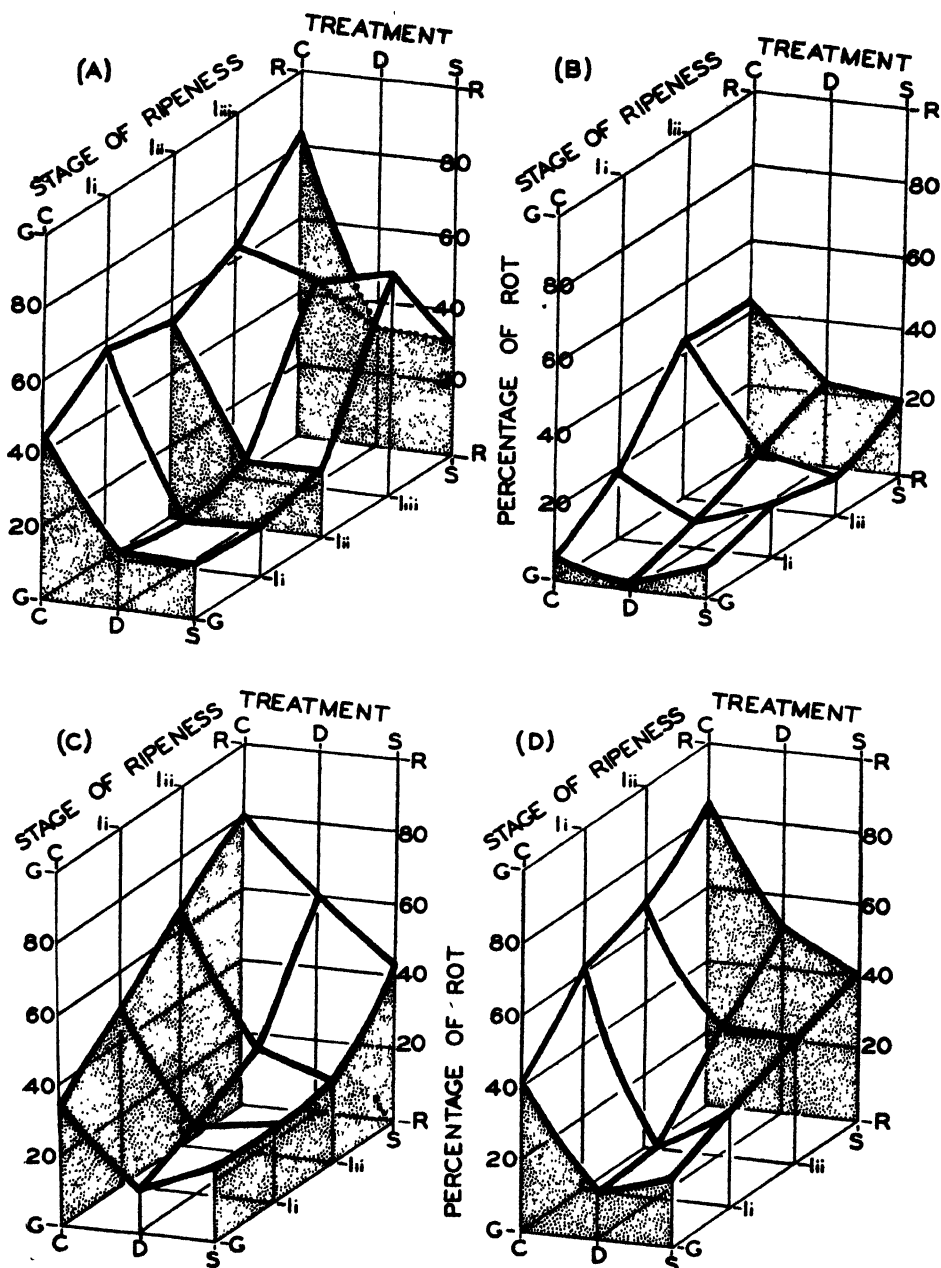
FIGURE 2.   Incidence of rot, in relation to treatment and stage of ripeness at harvest, in 1933 in Elberta peaches: (A) examined at Saskatoon; (B) at Winnipeg; (C) and (D) at St. Catharines.   Stages of ripeness and treatments as in Figure 1. See also Table 2

at room temperature and only one week after harvest, except where there was a 24-hour delay in cooling (Iiii, Figure 2, A).

Experiments in 1934

In and after 1934 a wider variety of spray materials was used, mainly for the purpose of comparing the more recently introduced pre-harvest sprays, such as Flotation sulphur, Bartlett's pre-pick, etc., with the older wettable sulphurs such as Koloform, etc. which have to be applied at a longer interval before harvest because of their tendency to mark the fruit.

The 1934 Rochester shipments (Figure 3, A) demonstrate not only the relative efficiency of the several fungicides under different conditions, but also the importance of those conditions. Neither shipment received the best treatment in transit, since the one, the day after picking, went in an iced car to Brandon and thence uncooled to Winnipeg, while the other, immediately after packing, went in an iced car to Yorkton and thence, uncooled to Saskatoon In spite of this treatment, the latter, except for the checks, was in a satisfactory condition a week after arrival, while the former had approximately the same rot development as the lot held at St. Catharines about the same number of days after harvest, but 8 days longer at room temperature (Table 3). The higher incidence of rot in the Winnipeg shipment may have been in some measure due to the rain which occurred the day before picking (Figure 6) but, in the light of the 1933 experience, the delay in getting the fruit cooled (Table 3) was, in all probability, mainly responsible.
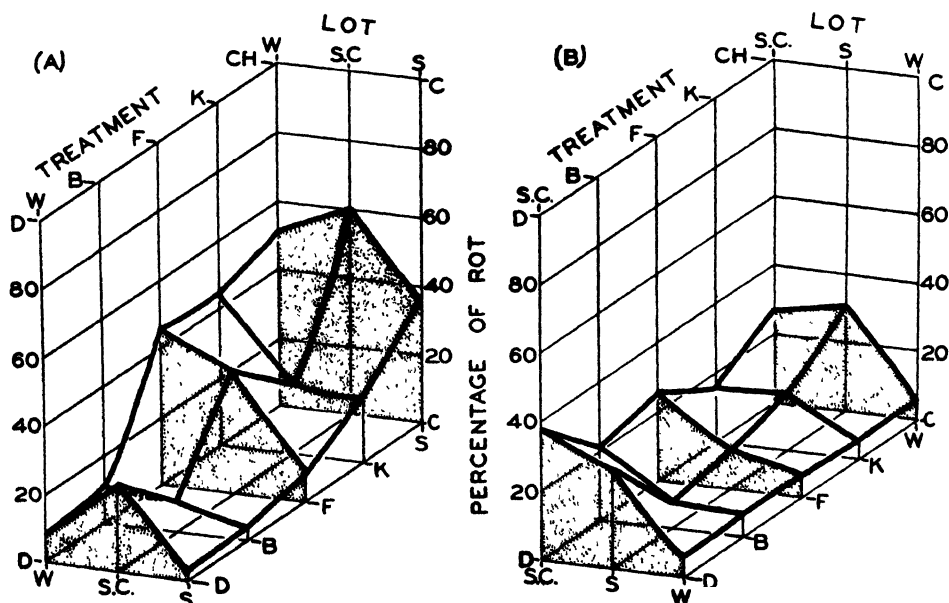
FIGURE 3. Incidence of rot, in relation to treatment and post-harvest conditions in 1934: in (A) Rochester peaches, intermediate stage of ripeness; (B) Elberta peaches, three stages of ripeness combined. Treatments: C, check; K, sprayed with Koloform; F, with Flotation sulphur; B, with Bartlett's pre-pick; D, dusted with sulphur. Lots: SC, examined at St. Catharines; W, at Winnipeg; S, at Saskatoon. See also Table 3.

TABLE 3.—DATA RELEVANT TO THE PICKS OF ELBERTA AND ROCHESTER PEACHES IN 1934
(SEE FIGURE 3)

Reference in Fig. 3	Date of		Observations			Room temp. (°F)	Remarks
	Pick	Arrival	Date	Days after			
				Harvest	Arrival		
Rochester A (W)	Aug 20	Aug. 29	Sept. 4	15	6	58–70 (62)	Put in iced car Aug 21, for Winnipeg, via Brandon.
A (S.C.)	Aug. 22	Aug. 22	Sept. 5	14	14	60–76 (65)	Held at St. Catharines.
A (S)	Aug. 22	Aug. 31	Sept. 7	16	7	56–70 (61)	Put in iced car for Saskatoon via Yorkton, Aug. 22.
Elberta B (S.C.)	Sept. 13	Sept. 13	Sept. 27	14	14	58–78 (66)	Papers damp in dusted lots. Humidity very high shortly after harvest.
B (S)	Sept. 13	Sept. 23	Oct. 1	18	8	50–71 (65)	Shipped to Saskatoon via Regina, Sept. 13.
B (W)	Sept. 10	Sept. 14	Sept. 21	11	7	59–72 (66)	Shipped to Winnipeg Sept. 10. Car iced only at loading.

The shipment of Elberta peaches to Saskatoon underwent treatment almost identical with that of the corresponding Rochester shipment, and, except for the dusted lots, with much the same results (Figure 3, B; Table 3). The failure of the dust to control brown rot was probably due to the intervening rains (Figure 6).   In the case of the Winnipeg consignment, as the car was iced only once at loading and not en route, the temperature during the latter part of the journey was undoubtedly higher than could be desired.   It is also exceedingly probable that, because of the melting of the ice and the influx of warmer air, the relative humidity in the car was abnormally high during the same period.   The 1935 Elberta shipment to Winnipeg (Figure 4, B; Table 4) travelled under similar conditions, except that it was loaded into the refrigerator car two days after harvest.   Since, in both instances, there was little difference in the amount of rot in the various samples it is suggested that prolonged periods of high relative humidity tend to render the fungicides ineffective.   This suggestion receives a certain amount of support from the behaviour of the Elberta peaches held at St. Catharines in 1934 (Figure 3, B) which were subjected to very high humidity during the first few days after harvest (cf. Figure 6).

Experiments in 1935

So far as the various lots of Rochester peaches were concerned (Figure 4, A) there was little to choose between the different fungicides employed, as the disease was evidently easy to control in 1935.   A comparison of the

checks in the lots, both of Rochester (Figure 4, A) and of Elberta (Figure 4, B) held at St. Catharines would indicate that the second pick has a tendency to be more susceptible to rot than the first.   In spite of this increased susceptibility, the incidence of rot was considerably higher in the Rochester shipment to Saskatoon than in that to Winnipeg, as a result of the delay between picking and cooling in the former (Table 4.)   The difference is all the more striking if the strictly comparable lots are considered together, that is, the Winnipeg consignment and the second pick at St. Catharines on the one hand, and the Saskatoon shipment and first pick at St. Catharines on the other (Figure 4, A).   The same effect appears again in the picks of Elberta (Figure 4, B).
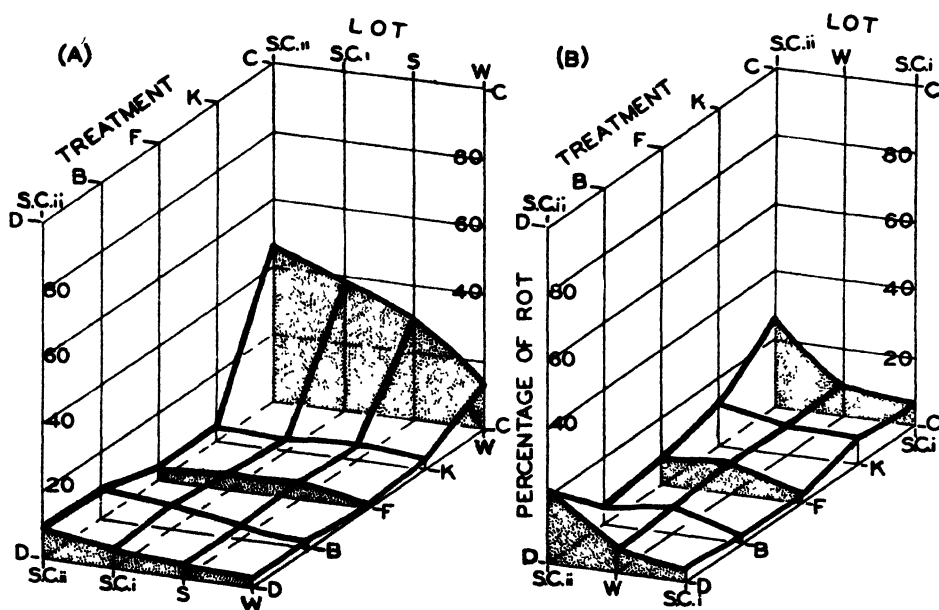
FIGURE 4.   Incidence of rot, in relation to treatment and post-harvest conditions in 1935: in (A) Rochester peaches, intermediate stage of ripeness; (B) Elberta peaches, three stages of ripeness combined.   Treatment and lots as in Figure 3.   See also Table 4.

Experiments in 1936

Because of the comparative drought throughout the growing season of 1936 (Figure 6) brown rot was of such minor significance in that year that no conclusions could be drawn from the experiments, beyond the obvious one that there is a marked positive correlation between the incidence and severity of brown rot and precipitation during the growing season, even when the rainfall is high only in the early summer (cf. 1935, 1936 and 1937, Figure 6).

Experiments in 1937

The incidence of rot in relation to the time factor and to treatment is illustrated in Figure 5.   These curves are typical of those obtained in previous years when the amount of rot appearing at successive observations
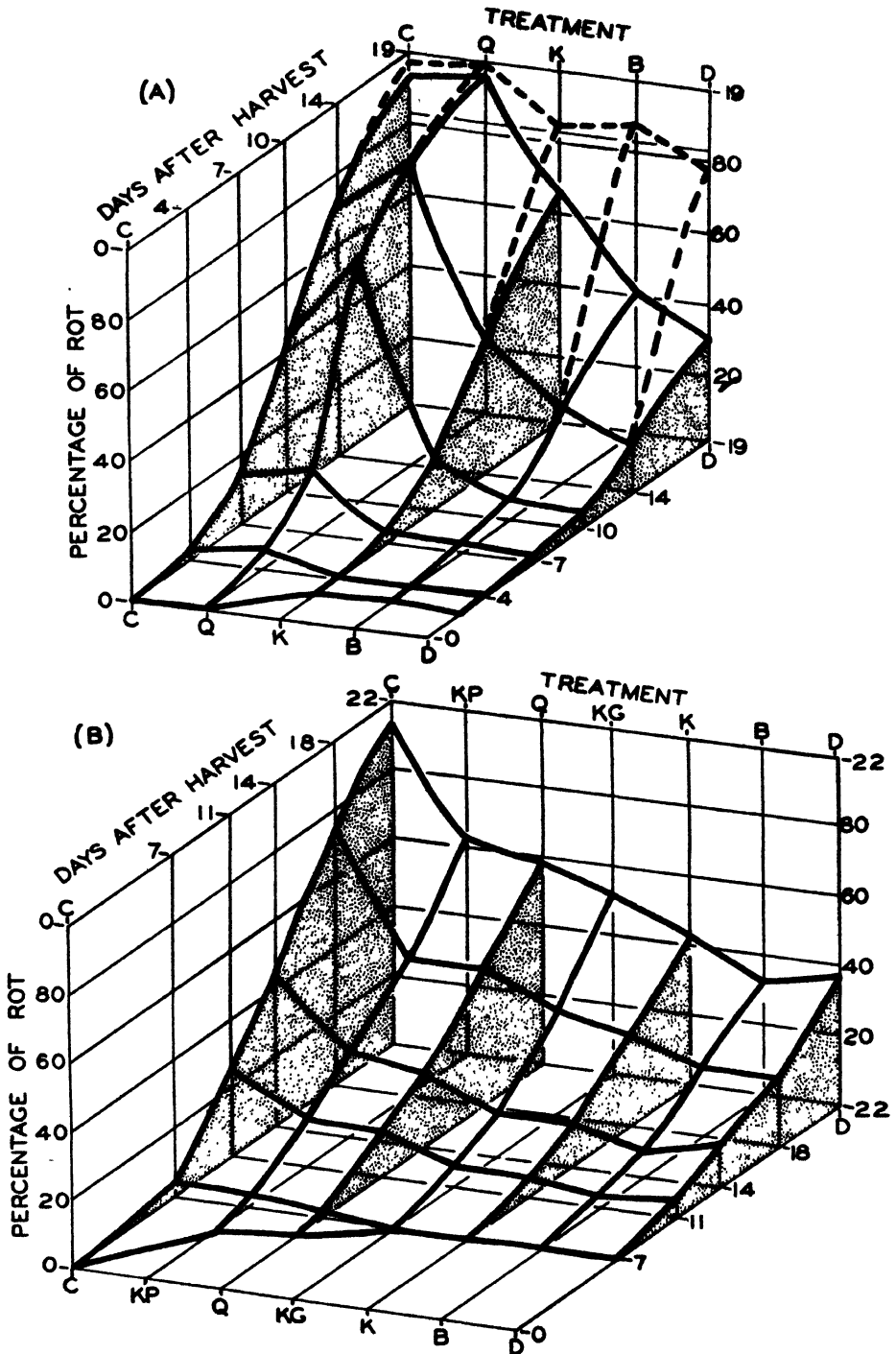
FIGURE 5. Incidence of rot, in relation to treatment and the time-factor, in 1937: in (A) Rochester peaches; (B) Elberta peaches. Treatments as in Figures 3 and 4, with the addition of KG, Kolofog extra; KP, Kolopick, and Q, Qua-sul. See also Table 5.

TABLE 4.—DATA RELEVANT TO THE PICKS OF ROCHESTER AND ELBERTA PEACHES IN 1935
(SEE FIGURE 4)

Reference in Fig. 4	Date of		Observations			Room temp., (°F.)*	Remarks
	Picking	Arrival	Date	Days after			
				Harvest	Arrival		
Rochester							
A (SCii)	Aug. 29	Aug. 29	Sept. 12	14	14	58, 60, 64, 61, 60 (60)	Second pick held at St. Catharines.
A (SCi)	Aug. 27	Aug. 27	Sept 10	14	14	64, 58, 63, 62, 60 (61)	First pick, held at St. Catharines.
A (S)	Aug. 27	Sept 3	Sept. 10	14	7	65, 64, 63 (64)	In iced car Aug. 28 for Saskatoon.
A (W)	Aug 29	Sept. 5	Sept. 12	14	7	66, 61, 61 (61)	In iced car Aug. 29 for Winnipeg
Elberta							
B (SCii)	Sept 20	Sept 20	Oct 3	13	13	63, 60, 56, 49, 49 (56)	Second pick held at St. Catharines
B (W)	Sept 17	Sept 25	Sept 30	13	5	63, 68 (66)	In iced car Sept 19 for Winnipeg, car iced once only
B (SCi)	Sept 17	Sept 17	Sept 30	13	13	67, 63, 60, 56, 49 (60)	First pick held at St. Catharines

* Mean temperature of successive 3-day intervals, (mean temperature for full period in brackets)

was plotted against time elapsed after harvest. In the curves for the Rochester variety (Figure 5, A) the broken lines indicate the total number of peaches affected with rot in each lot, while the solid lines include adjustments to allow for "nesting" and for the secondary rots accompanying senile breakdown. The solid line, then, represents individual infections, chiefly those of brown rot, though including those caused by *Rhizopus nigricans*.

While it is true that there was a markedly higher percentage of rot in Rochester peaches 19 days after harvest than there was in Elberta after 18 days, a study of the temperature obtaining during the respective holding periods (Table 5) would suggest that the incidence of rots in these 2 varieties is influenced as much by meteorological conditions just before and after harvest as by inherent differences in susceptibility, which may be less than is generally supposed.

ENGLISH SHIPMENTS

Except in 1933, the peaches consigned to England were packed in wood wool in single layer boxes wired in pairs. This method of packing greatly reduced damage from bruising in transit. Usually the packs were expressed to Montreal and there transferred to the refrigerated hold of the ship, where they were kept at a temperature of 33° to 36° F. until arrival at London or Liverpool.

TABLE 5.—DATA RELEVANT TO PICKS OF ROCHESTER AND ELBERTA PEACHES IN 1937, EXAMINED AT ST. CATHARINES

(see FIGURE 5)

Reference in Fig. 5	Date of pick	Mean daily temperature (°F.), (approximate temperature of packing house) — Days after harvest																							Mean
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
(Rochester) A	Aug. 20	78.3	78.0	66 6	64.0	67.5	68 9	71.6	74 9	74 0	70 9	74.1	75 6	78 8	79 7	81 2	75.6	61.9	56.2	62.0	69.2				71.5
			74.3		66 8		73 5		73 5		79.9				64 5			65.6								
(Elberta) B	Sept. 16	57.2	52 0	54.3	54 2	50 7	55 4	55 3	64 4	72 4	75 1	58 3	49.3	49.6	54 4	52.8	66 4	56.8	50 1	55 6	63.1	71.8	54.3	42.8	57.2
			54 5		53 5		64 0		60 9		52 3				57.8			63.5				48.5				

The results need not be discussed in detail as in most years very little rot occurred in any lot except the check, so that, except in 1937 when the data agreed very well with those obtained in St. Catharines, it was not possible to arrive at any conclusion concerning the relative efficiency of the fungicides used. The chief interest in the shipments to England was the demonstration that, with proper treatment and careful handling, brown
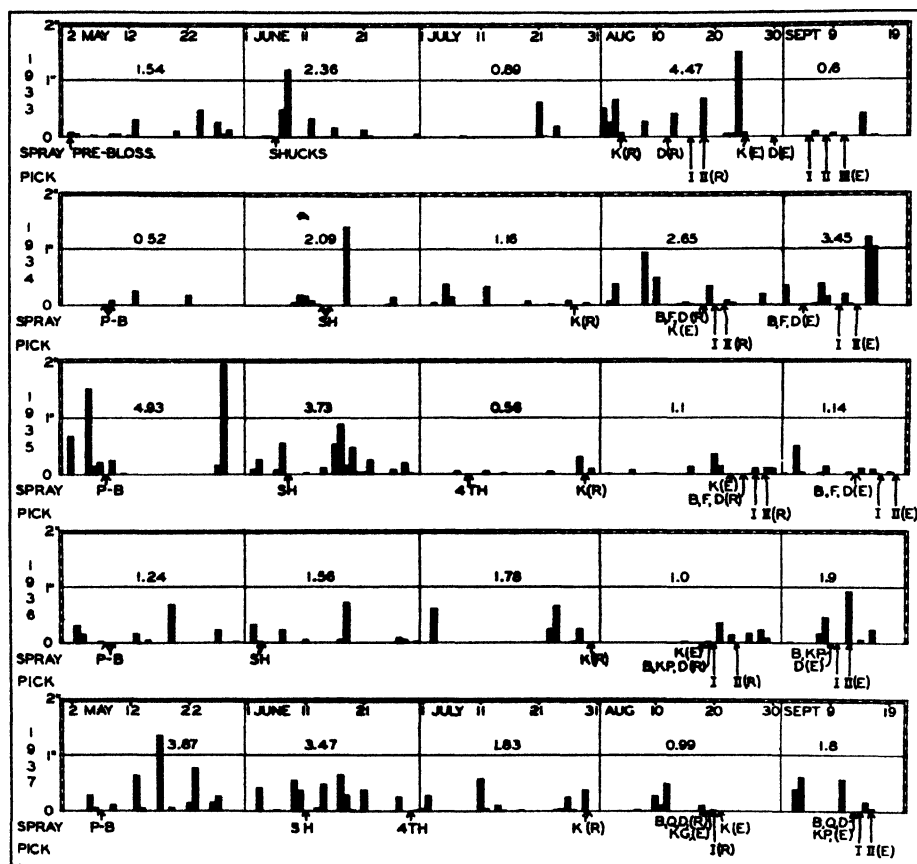
FIGURE 6. Distribution of rainfall and monthly precipitation during the growing seasons of 1933–37 inclusive, and dates of spraying and of harvest. P.B., pre-blossom spray; Sh., shucks spray, K and KG indicate the date of the last application of Kolo-form and Kolofog extra for the variety specified (applications of other materials were made in their respective plots at the same time). KP, B, Q, D indicate, for the variety specified, the date of last application of Kolopick, Bartlett's pre-pick, Qua-sul and dust respectively. (R) indicates the variety Rochester, and (E) the variety Elberta.

spot can be satisfactorily controlled in a journey of several thousand miles covered in a period of slightly over 2 weeks. In 1937, also, a commercial shipment of more than 50 boxes of sprayed Elberta peaches from the labor-atory orchard was packed by a local concern and exported to various points in the British Isles. As a result of a questionnaire enclosed in each box, favourable reports were received concerning the absence of rot in this consignment.

DISCUSSION

Throughout the experiments recorded here, *Sclerotinia fructicola* has been the predominating cause of decay in harvested peaches. *Rhizopus nigricans*, however, appeared in many of the packs and caused considerable damage. *R. nigricans* differed from *S. fructicola* in that it spread more rapidly from peach to peach because of its more luxurious aerial growth. In this way it gave rise to "nests" of rotted fruit centred about the one in which infection first occurred. This habit would obviously tend to make *R. nigricans* a potentially more serious menace than *S. fructicola*. The latter was also capable of producing "nested" rots, but usually only in instances where the pack remained undisturbed for a longer interval than the 3 or 4 days between examinations. That *R. nigricans* remained of secondary importance was probably due to the fact that new baskets were used during picking and that, in grading, the fruit was not spread out on the packing house bench, but was either sorted from the basket or transferred to clean trays. Furthermore, the peaches were generally packed in new lugs, except in 1937, when the used lugs were first scrubbed and then sprayed with wettable sulphur before being returned to use.

Rots caused by other fungi, such as *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., etc., also attacked the fruit, frequently around the stem insertion, but only when the peaches were obviously near the end of their storage life and long since past the stage of edibility. Such rots were therefore considered as secondary and coincidental with a loss of resistance indicative of the initial stages of physiological breakdown. At the stage of development at which the secondary rots made their appearance, the skin of the peach ruptured very easily and could be "slipped" in a crumbling condition, due to the loss of cohesion between its constituent cells and between those of the skin and the flesh.

It is well known that with increasing ripeness, peaches become more susceptible to rot, a correlation apparent from the sigmoid character of the curves illustrating the increase in the amount of rot with the passage of time (Figure 5). From another point of view, this tendency is also reflected in the post-harvest behaviour of the disease in different lots, since it is indicated (Figures 1 and 2) that, at given time within the period of observation, the incidence of rot was higher in the riper than in the greener peaches, both treated and checks. As already mentioned in connection with the 1935 experiments (Figure 4), there was considerable evidence that the first picks were not so subject to rot as the later ones. This not only seems to be another manifestation of the effect of the stage of maturity upon susceptibility, but also suggests that the maturation and ripening processes may take place more rapidly than the changes of colour would indicate. On the other hand, the differences between successive picks may also be explained on the basis that the maturity grades into which each pick was divided are arbitrary and may not be comparable with similar grades in other picks. The first hypothesis, however, has this to support it, that, in tests made with the aid of colour charts and a pressure tester, peaches on the tree were found to soften more rapidly than they changed colour. In effect, this means that, in a later pick, peaches at a given stage as denoted by ground colour would be softer than peaches of a similar colour in the preceding pick. This applies more particularly to the

"green" and "intermediate" grades of the experiments, as there was a reasonably close correlation between pressure and colour when the fruit was immature, or only just mature, and again when it was almost ripe.

Although there were some indications of increased decay as a result of careless handling (Figure 1 and 2), it was observed that bruises did not markedly contribute to the greater incidence of decay unless the skin of the fruit was broken at the same time. However, since the bruises themselves constitute a serious form of wastage and since there is no guarantee against breaking the skin, careful handling of peaches during picking and the later operations is imperative. It is also of interest in this connection that skin punctures of long standing, that were closed over at harvest time, were no more susceptible to rot than unbroken skin.

In consequence of spraying or dusting there was usually a marked reduction in the development of rot in peaches at various grades of ripeness (Figures 1 and 2) but it should be pointed out that none of the sprays or dusts, under the conditions of the experiment, gave absolute control. The chief importance of these preventive measures seems to lie in their delaying the onset of fungal wastage long enough to allow the sale and consumption of the fruit in a satisfactory condition. Moreover, the use of suitable fungicides, in combination with judicious post-harvest treatment in the matter both of handling and of carrying temperatures, permits the shipping and marketing of peaches picked ripe enough to develop to good quality and does away with the necessity, more or less imaginary, of shipping immature fruit.

Some differences were noted in the effectiveness of the fungicides used in these experiments (Figures 3, 4, 5). In this regard, the sulphur content of the spray mixture, the size of the sulphur particles, the time of application, the adhesiveness and, in the case of one of the materials, the solubility of the residue, each play their part. Generally speaking, the pre-harvest sprays, especially those with higher sulphur content, gave the best and most uniform results. Materials which, because of their tendency to mark the fruit, had to be applied 3 to 4 weeks before harvest, were almost as effective, though more variable, under most of the conditions encountered. This would suggest that such materials adhere well but may be less efficient than the pre-harvest sprays under critical conditions, because of the loss of coverage occasioned by the rapid growth of the peach fruit, during the last few days before harvest. Dust was, in many cases, as good as or even slightly better than the pre-harvest sprays, but in others much worse. This lack of uniformity and of reliability in the efficiency of the dust could be accounted for, in most instances, by the fact that the dust was more readily washed off by rain than were the sprays.

In those instances where the packs were transferred to low temperatures, 45° F. or lower, within 6 or 8 hours after picking, little or no rot appeared until after they were returned to higher temperatures. On the other hand, where they were held for an additional 24 hours before being cooled, the incidence of rot at the end of the period of low temperature was, as a general rule, about the same as that in lots not cooled at all. That is to say, the delay very often had the effect of nullifying the effects of cooling. Sometimes, in the 1935 shipment of Elberta to Saskatoon and in the 1937 experiments, for example, there were no differences between the lots cooled

immediately after packing and those cooled 24 hours later. Whether or not the effect of the delayed cooling became apparent was evidently dependent on the conditions prevailing in the interval in question. The beneficial effects of prompt cooling, as indicated by Brooks and Cooley (4), are undoubtedly due to the fact that infection does not occur readily, if at all, at low temperatures, even though, once it has become established in the fruit, the fungus is capable of growing within the same range of low temperature. Since peaches take some time to cool in refrigerated cars (4), because the bulk of warm fruit is large in relation to the cooling capacity of the car, pre-cooling of peaches destined for long hauls is desirable, if facilities are available, and particularly if the weather has been unfavourable.

It should be observed that, during the 5 seasons covered by these investigations, conditions immediately preceding and following the harvest of the experimental material were not favourable for a serious outbreak of the disease, either in the orchard or in picked fruit. The control measures used in these experiments then, appear to be adequate for average seasons in Ontario. Under abnormal or more unfavourable circumstances, it becomes imperative not to overlook any precautions, either before or after harvest, if the fruit is to be marked without substantial loss.

RECOMMENDED CONTROL MEASURES

1. Practise strict sanitation in the orchard by cutting out blighted twigs at pruning time, or in early summer, and by removing mummied fruits from the tree after harvest. Mummies should be either gathered and burnt, or ploughed under.

2. Adopt a summer spray programme which should include a pre-blossom spray for the control of blossom blight, a "shucks" spray where necessary, for the control of scab and curculio, a spray or dust for brown rot, three or four weeks before harvest and again at harvest time, particularly if the weather is warm and damp at that time. An extra spray two or three weeks after shucks may be necessary in wet seasons, for brown rot of green fruits and mildew.

3. At harvest and during packing, handle the fruit carefully in order to prevent breaking of the skin or bruising. Finger bruises can be avoided by picking with a "cupped" hand instead of grasping the fruit with fingers and thumb.

4. Do not handle decaying peaches while picking for the market. If decayed fruits do find their way to the packing house, they should not be allowed to go through the grader or to contaminate the packing bench.

5. It is advisable to use clean utensils and containers during picking and packing operations. If the packing bench or the grader should become contaminated through contact with rotten peaches, they should be scrubbed with a strong solution of washing soda or a 10% solution of copper sulphate.

6. In preparing for long distance transportation, cool the fruit promptly after packing, preferably within 12 hours of picking. Do not cool first and pack afterwards because of the tendency of the chilled fruit to "sweat" on contact with warm air. In any case, low temperatures should be maintained until the shipment reaches its destination.

7. Precooling may not be necessary in dry seasons, but should be practised, if possible, when conditions at harvest are favourable to brown rot.

SUMMARY

1. In order to test the efficiency of various pre-harvest fungicidal treatments and the effects of other factors and conditions as related to the incidence of brown rot during long-distance shipping, numerous packs of peaches of the varieties Rochester and Elberta were shipped overland to the Canadian West and overseas to England, at which destinations they were examined upon arrival and at intervals thereafter.   Duplicates of these shipments were given corresponding examinations at the St. Catharines laboratory.

2. While the riper grades of peaches were more susceptible to rot than the green, it was found possible to delay the incidence of rot in the riper grades long enough to permit marketing.

3. For either variety, the later picks were somewhat more susceptible to rot than were the first picks of the season.

4. A number of different forms of wettable sulphur were used in the experiments.   In general, those sprays which could be applied immediately before harvest gave more uniform results than those which had to be applied 2 or 3 weeks earlier.   Dusting with sulphur was also satisfactory except when rain intervened before harvest.

5. Wastage in the form of bruises and, to a lesser extent, of rots was reduced by careful handling during picking and packing operations.

6. The incidence of rot was usually much higher when peaches were cooled after a delay of 24 hours than when cooled shortly after picking.

7. In the case of shipments in which the car was iced only on departure there was little difference in the development of rot in treated and non-treated peaches.

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DETERMINATION OF NITRITE, NITRATE, AND CHLORIDE IN CURED MEAT AND CURING PICKLE[1]

By W. H. White[2]

Abstract

An extract suitable for the quantitative determination of nitrite, nitrate, and chloride in cured meat was prepared by freezing and thawing the sample, followed by extraction with hot water. The sulphanilic acid-α-naphthylamine hydrochloride method, applied to the photoelectric colorimeter, was suitable for the determination of nitrite in meat extract and curing pickle. A number of factors that affect this reaction were investigated. The nitrate content of cured meat and curing pickle was determined by the phenoldisulphonic acid method, slightly modified and applied to the photoelectric colorimeter. The chloride content of meat extract was determined, either by direct titration with potassium chromate as indicator, or by Volhard's procedure, after the removal of protein by ignition or wet oxidation. The latter method should be used if accuracy within 5% is desired. Direct titration was satisfactory for the determination of chloride in curing pickle.

The precision of the above methods is illustrated by the following average deviations of individual determinations from their means, as computed from 25 or more duplicate determinations, and expressed as a percentage of the amount present, for meat and pickle respectively: chloride, ±0 20% and ±0 02%; nitrate ±0 70% and +0 30%; nitrite, ±1 7% and ±0.1%.

Introduction

Preliminary to a survey of Wiltshire curing practice in Canada, an extensive study was made of methods for determining the nitrite, nitrate, and chloride content of cured meat and curing pickle. Many of the available procedures were found to be too laborious, or not adaptable to routine analysis, while others lacked precision or accuracy. A study was therefore undertaken with the object of developing procedures applicable to routine work, and capable of giving satisfactory reproducibility and accuracy.

Methods of the A.O.A.C. (8, pp. 354–357) for the determination of chloride, nitrate, and nitrite in cured meats require separate portions of the sample for each determination. When all three components are to be determined in the same sample, it is desirable that one extract should serve for the complete analysis. Attention was therefore given to suitable methods for preparing such an extract.

Since the concentrations of nitrite and nitrate in a cured meat extract of this kind are rather low, considerable attention was given to colorimetric methods sensitive to small quantities of these constituents. Several of the

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difficulties inherent in such procedures were overcome by the use of a photo-electric colorimeter. This paper describes a new method for extracting salts from cured meat, and advantageous modifications of existing procedures for determining the components of this extract and of curing pickle.

Preparation and Extraction of the Sample

Cured Meat

A number of possible methods for suitably preparing the sample for aqueous extraction were investigated. There is evidence from related studies that extraction is facilitated by grinding the sample with sand (11) or by freezing and thawing it (2). The effect of these two treatments was studied by passing a sample of Wiltshire bacon through a food chopper adjusted for fine grinding, until it was thoroughly ground and mixed. This material was then divided into four sub-samples, and treated as follows: (i) untreated; (ii) minced and ground with a coarse silica sand; (iii) minced, frozen, and thawed; and (iv) minced, frozen, thawed, and ground with sand. Each sub-sample was again divided for extraction with hot (boiling) or cold water. After adding 100 ml. of water to 15 gm. of each sample, the whole was shaken for 2 hr., centrifuged and the extract decanted. Nitrite and chloride determinations on each extract gave a measure of the completeness of the extraction. The average values for complete duplicate determinations are given in Table I.

TABLE I

EFFECT OF PRELIMINARY TREATMENT OF THE SAMPLE AND TEMPERATURE OF THE WATER ON THE EXTRACTION

Procedure	$NaNO_2$, %		$NaCl$, %	
	Cold extraction	Hot extraction	Cold extraction	Hot extraction
Minced	0 0022	0 0023	3 36	3.51
Minced and ground with sand	0.0021	0 0025	3 43	3 63
Minced, frozen, and thawed	0.0023	0.0027	3.69	3.70
Minced, frozen, thawed, and ground with sand	0 0025	0 0026	3.58	3 69

The results show that mincing, freezing, and thawing the sample give the best extraction of the salts. Although grinding with sand is better than mincing alone, no definite advantage is gained by its use in the freezing and thawing procedure. More nearly complete extraction was obtained with hot than with cold water.

The extraction proper may be accomplished by two more or less distinct methods. The first may be termed "complete" extraction, and involves extraction by shaking with successive portions of the hot solvent until the process is complete. The decanted solutions are combined, made up to volume, and an aliquot analyzed. The second method may be termed

"equilibrium" extraction, since the sample is placed in a volumetric flask with hot water, shaken for a given period, made up to volume, and a suitable aliquot taken for analysis. Such a method assumes that an equilibrium will be attained in which the quantity of salts contained in unit volume occupied by the meat will be the same as that in unit volume of aqueous extract. In this method correction for volume occupied by the dry matter may be made, but is usually negligible.

A comparison of the two procedures showed that five extractions were required for the complete removal of the salts, and that equilibrium was reached after shaking for 2 hr. Although "complete" extraction appears to have a sounder basis, the "equilibrium" method involves fewer manipulations, and is consequently more suitable for routine analysis.

A number of miscellaneous experiments on other types of extraction, and on factors that might affect extraction, were conducted. Refluxing meat samples with water gave lower results than the above procedure, although the liquid was kept definitely alkaline in order to prevent the loss of nitrite (4). Adjusting the pH of minced, frozen, and thawed samples with lactic acid or ammonium hydroxide had no beneficial effect on the "equilibrium" method of extraction.

The following procedure was therefore adopted for the extraction of salts from cured meat:

Lean meat from the sample to be analyzed was thoroughly minced, and mixed by several passages through a food chopper adjusted for fine grinding. After freezing and thawing, a 10-gm. sample was weighed into a 100-ml. beaker, a small quantity of cold water added, and the mixture worked into a paste. This was transferred to a 200-ml. wide-neck volumetric flask, and a sufficient quantity of boiling water added to bring the final volume to approximately 150 ml. The flask was stoppered, and shaken vigorously for 2 hr. in a shaking machine equipped with a steam chest to maintain the flask at a temperature of 80° C. or higher. The contents of the flask were then brought to room temperature within a period of one-half to one hour, made up to volume, shaken thoroughly, and filtered through a large fluted filter. Portions of this one extract, after suitable dilutions, served for nitrite, nitrate, and chloride determinations.

For the most part, clear extracts are obtained which do not require the use of any protein precipitant as a clarifying agent. The results of a large number of determinations have shown the method to be quite satisfactory, both in its applicability to routine work, and in its precision and accuracy. A comparison of the results given by this method with those obtained by the standard procedures of the A.O.A.C. (8, pp. 354–357) will be given later.

Curing Pickle

Since curing pickle already contains the salts to be determined in the form of a solution, no preliminary treatment of the sample is necessary.

Application of the Photoelectric Colorimeter to the Determination of Nitrite and Nitrate

Although the procedures to be described later are suitable in most cases for visual colorimetry, they have been adapted especially to the photoelectric colorimeter. The comparison of colour intensities by such an instrument eliminates many of the objections levelled at colorimetric methods, such as the necessity of preparing a number of standards, their possible variation from day to day, and the inadequacy of the human eye for accurate comparison of varying intensities of the same colour. The photoelectric colorimeter used in these investigations was that designed by Evelyn (3). If the reaction obeys the Lambert-Beer law, a constant relating the transmitted light and the concentration of the constituent may be calculated. If it does not obey this law, a calibration chart must be prepared by plotting values for concentration against corresponding galvanometer deflections. Details necessary for the use of the photoelectric colorimeter in these determinations are given below.

Spectrophotometric examination of the red colour developed in the sulphanilic acid-α-naphthylamine hydrochloride method for the determination of nitrite indicated that a Rubicon No. 520 filter, transmitting 95% of the incident light in the range 4950Å to 5500Å, was suitable. The results of a complete series of triplicate determinations on a number of standard solutions of sodium nitrite varying in concentration from approximately 1×10^{-3} to 1×10^{-4} mg. per ml. of solution gave an average value of 1.0 for K_1, and showed that the Lambert-Beer law was applicable (Table II).

TABLE II

VALUES OF CORRECTED GALVANOMETER DEFLECTION AND OF K_1 FOR THE DETERMINATION OF NITRITE

Conc. of NaNO$_2$, (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	K_1	Conc. of NaNO$_2$, (mg. $\times 10^{-3}$ per ml.)	Corrected galvanometer deflection	K_1
1.37	6.00	0.89	0.319	48.00	1.00
0.910	13.25	0.97	0.296	50.50	1.00
0.683	20.75	1.00	0.273	52.75	1.02
0.637	23.25	1.00	0.251	56.50	0.99
0.546	28.75	0.99	0.228	59.25	1.00
0.501	32.50	0.97	0.182	65.00	1.03
0.455	35.00	1.00	0.091	80.00	1.06
0.410	38.50	1.01	0.061	86.25	1.05
0.364	43.50	1.00	0.036	91.50	1.08
0·347	45.45	0.99			

A Rubicon No. 420 filter, transmitting 95% of the incident light in the region 3800Å to 4600Å, was used for the intensity measurements of the yellow colour developed in the phenoldisulphonic acid method for nitrate. Preliminary investigations indicated that the method gave low values compared to other procedures, and did not obey the Lambert-Beer law (possibly

due to occlusion of nitrate by the bulky precipitate formed). In order to overcome these difficulties somewhat, a calibration curve was prepared with standard sodium nitrate solutions, to which sodium chloride was added in order to simulate, in part, the conditions existing in meat extract and curing pickle. The results are illustrated graphically in Fig. 1 for the range from 0 to 1.00 mg. of sodium nitrate, and for a colour dilution of 100 ml.
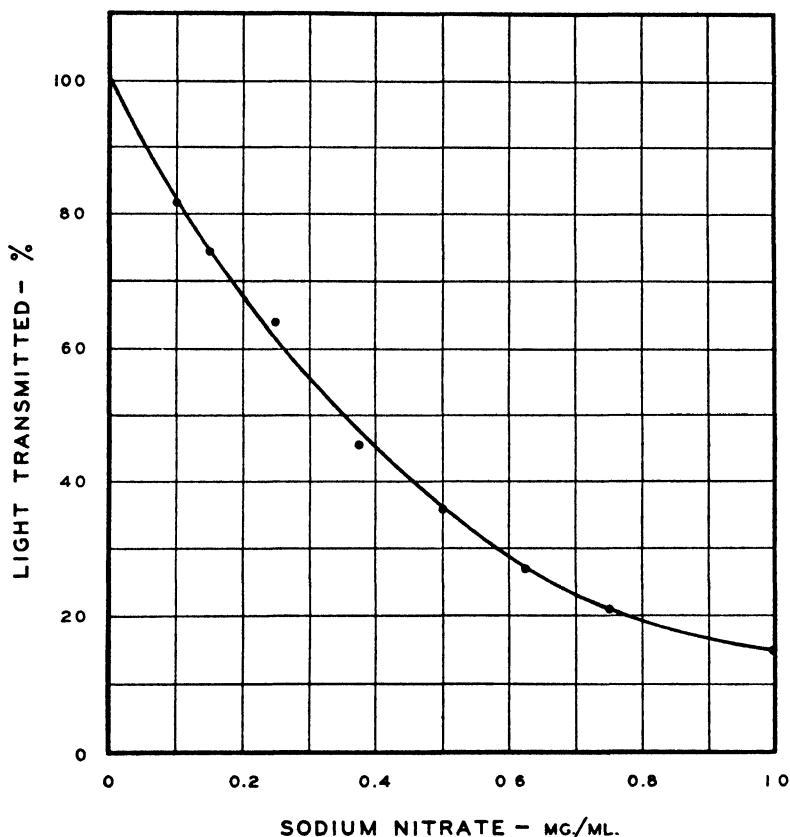
FIG. 1. *Calibration curve for the determination of nitrate by the phenoldisulphonic acid method.*

DETERMINATION OF NITRITE

Cured Meat

Of a number of colorimetric methods available for the determination of nitrite, that proposed by the A.O.A.C. (8, p. 506) was selected and found to be entirely satisfactory. To 10 ml. of meat extract, diluted to contain approximately 1.0×10^{-3} to 1.0×10^{-4} mg. of sodium nitrite per ml. of solution, was added one drop of concentrated hydrochloric acid, one ml. of a solution of sulphanilic acid, and one ml. of α-naphthylamine hydrochloride, and the tube shaken. The blank solution was prepared in the same way, but the sulphanilic acid was omitted.

The effect of a number of factors on rate of development and maximum intensity of the colour was investigated. The rate was studied by making readings of the galvanometer deflection against time for a number of solutions varying in concentration of sodium nitrite from 0.036×10^{-3} mg. to 1.37×10^{-3} mg. per ml. of solution. The results for each of the 19 concentrations indicated that the rate was very rapid during the first 5 min., and that readings made 15 min. after the addition of the reagents corresponded to maximum colour intensity. It was observed that, for the more dilute solutions, the colour had faded only very slightly even after a period of 24 hr. However, for the more concentrated solutions, the intensity had decreased considerably at the end of this period. A few of the typical curves obtained are shown in Fig. 2.
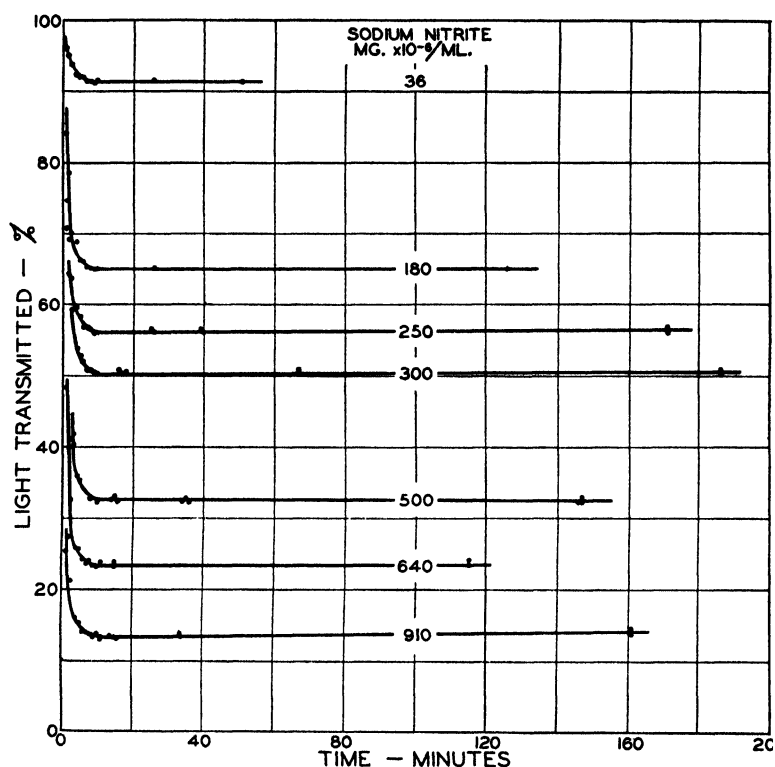
FIG. 2. *Colour development at various concentrations of nitrite in the sulphanilic acid-α-naphthylamine hydrochloride method.*

Other factors investigated were temperature, light, sodium chloride, pH, concentration of reagents, their age, and the method of their addition. The results are shown in Table III. The colour of a solution held in a steam bath developed more rapidly than that of one kept at room temperature. After reaching its maximum in 4 min., the intensity subsequently decreased, indicating that the chromogen was being either precipitated or destroyed.

Both the rate and maximum intensity of colour were increased by decreasing the intensity of incident light during colour development.

TABLE III

EFFECT OF A NUMBER OF FACTORS ON THE RATE AND FINAL INTENSITY OF THE COLOUR DEVELOPED IN THE SULPHANILIC ACID-α-NAPHTHYLAMINE HYDROCHLORIDE METHOD FOR NITRITE

Factor studied	Experimental details	Conc. of NaNO$_2$, (mg. $\times 10^{-3}$ per ml.)	
		Theoretical	Found
Temperature	Room temperature	0 185	0 185
	Heated on steam bath for 5 min.	0 185	0 181
	Heated on steam bath for 268 min.	0 185	0 095
Light	Sunlight	0 185	0 170
	Laboratory conditions	0.185	0.185
	Darkness	0 185	0 189
Sodium chloride	10% solution	0 185	0.186
	20% solution	0 185	0 187
	30% solution	0 185	0 187
pH	0 95	0 185	0 124
	1 82	0.185	0 185
	6 58	0 185	0 174
Addition of reagents	1 cc. of each, added separately	0 185	0 185
	1 cc., mixed	0 185	0 163
	2 cc., mixed	0.185	0.166
Concentration of reagents	1 cc. of each, added separately	0 185	0 185
	5 cc. of each, added separately	0 185	0 208
Age of reagents	Freshly prepared	0.185	0.185
	One week old	0 185	0 189

The presence of sodium chloride slightly increased the rate of colour development but had no effect, within experimental error, on the intensity of the fully developed colour. There is some indication that the observed increase in rate varies directly with the concentration of sodium chloride. The effect of pH was studied on three solutions adjusted to pH values of 0.95, 1.82, and 6.58, the second being the value used in all previous determinations. The rate of colour development was approximately the same for the two solutions of low pH, but was considerably retarded at the higher value. The final colour intensity was greatest for a pH of 1.82, less for 0.95, and least for 6.58.

Both the rate and final intensity of the colour are decreased by mixing the reagents prior to their addition. On a comparable volume basis, the results indicate that these two properties vary directly with the concentration of reagents employed. A solution of α-naphthylamine a week old, although considerably discoloured, gave results strictly comparable with those obtained with a freshly prepared solution.

The procedure proposed here for extraction and determination of nitrite gave results approximately 1% lower than that of the A.O.A.C. (8, pp. 356–357), but of equally satisfactory precision.   The results of 25 duplicate determinations on smoked and unsmoked Wiltshire bacon, with nitrite concentrations ranging from 0.00023% to 0.013%, showed an average variation about their means of ±1.7%.

Curing Pickle

The method described above is applicable without further modification to the determination of the nitrite content of "pump", "cover", or "spent" pickle*.   The use of the photoelectric colorimeter makes it unnecessary to remove any protein present which may give colour to the pickle.   The results of analyses on 25 pickles of each type showed an average variation about the mean of ±0.1%, ±0.2%, and ± 0.1% for pump, cover, and spent pickle respectively.

DETERMINATION OF NITRATE

Cured Meat

The nitrate content of cured meat may be determined either gasometrically or colorimetrically.   Since the gasometric method requires a relatively large quantity of nitrate for accurate determination, its use would necessitate the preparation of a more concentrated extract than required for the determination of nitrite and chloride.   As this was not desirable, attention was given to colorimetric procedures.   Of the several possible methods, only three were thought to merit consideration.   Both the brucine (10) and phenoldisulphonic acid methods (8) have been applied to cured meat.   A more recently described method (7), depending on the reduction of nitrate to nitrite, and its subsequent determination, was thought to be applicable.   Preliminary studies, however, indicated that the brucine and nitrate-nitrite reduction methods were both unsatisfactory because of the inconsistency of the results obtained.

Difficulty was also encountered in obtaining satisfactory results with the phenoldisulphonic acid method described by the A.O.A.C. (8, p. 356).   A study of possible causes for the discrepancies observed confirmed a previous finding (1) that the solution should be alkaline during evaporation.   The addition of sodium hydroxide serves a further purpose in that any excess silver sulphate present is precipitated.   Nitrite interferes and is removed by oxidation to nitrate with potassium permanganate.   A suitable method for the preparation of the phenoldisulphonic acid reagent is described in (9, p. 633). The details of the suggested modification of this method as applied to cured meats are given below.

To a 25-ml. portion of the extract in a 100-ml. volumetric flask, one drop of sulphuric acid (1 : 10) is added, followed by 0.6% potassium permanganate solution, drop by drop, until a pink colour remains in the extract for approximately 2 min.   Chloride is precipitated with a saturated solution of silver

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* *The pickle injected into the sides is designated here as pump, the freshly prepared tank pickle as cover, and that removed from the tank after cure as spent.*

sulphate added in slight excess, as indicated by precipitation on the addition of a few drops of *N* sodium hydroxide (carbonate-free). Protein material is then precipitated by adding 2 ml. of saturated basic lead acetate solution, followed by a sufficient quantity of the sodium hydroxide solution to make the solution alkaline to litmus. The flask is shaken thoroughly after the addition of each of the above reagents, the solution made up to volume, shaken, and filtered through a coarse paper until clear. (The use of a fine filter paper will result in sufficient retention of nitrate to affect the results appreciably (5).) A suitable portion of the extract, containing 0.15 to 1.0 mg. of sodium nitrate is pipetted into an evaporating dish, and taken to dryness on the steam bath.

The residue is dissolved in 2 ml. of the phenoldisulphonic acid and, after standing for 10 min., diluted with 25 ml. of cold water. The solution is made definitely alkaline with concentrated ammonium hydroxide (about 10 ml.), and transferred to a Nessler tube graduated at 50 and 100 ml. The contents are made up to either volume, depending on the intensity of the colour, shaken, filtered through a fine paper, and a portion of the filtrate transferred to a comparison tube of the photoelectric colorimeter. The blank solution, used in the initial setting of the colorimeter, is prepared by adding the same quantities of ammonium hydroxide and water to 2 ml. of the phenoldisulphonic acid reagent. As the method determines both the nitrate and nitrite present, the percentage of nitrate is obtained by subtracting that for nitrite from the total. However, the nitrite content of Wiltshire bacon is usually quite low, and may be neglected if nitrate alone is being determined.

This method for the extraction and determination of nitrates gives quite satisfactory reproducibility. The results of 25 duplicate determinations picked at random from a large number of analyses in duplicate of Wiltshire bacon (both smoked and unsmoked) showed an average variation of ±0.73% about the mean.

Curing Pickle

The above described method is applicable to the determination of the nitrate content of a suitably diluted portion of curing pickle. In this instance a 0.2% solution of potassium permanganate is used for the oxidation of nitrite to nitrate. The analyses in duplicate of 25 pump, cover, and spent pickles showed an average variation about the mean of ±0.3%, ±0.2% and ±0.4% respectively.

This colorimetric method was compared with the Schlösing-Wagner gasometric procedure (8, pp. 355-356). The nitrate content of a pump, cover, and spent pickle was determined by each of the two methods. The results shown in Table IV indicate that the phenoldisulphonic acid method gives comparable results for pump, but lower values for spent and cover pickles. This is believed due to the occlusion of nitrate by the rather bulky precipitate formed by protein usually present in the last two types, but either absent, or present in a negligible quantity, in the first. The colorimetric

procedure gives closely reproducible results, but is somewhat lacking in accuracy.

TABLE IV

COMPARISON OF THE COLORIMETRIC AND GASOMETRIC
METHODS FOR THE DETERMINATION OF NITRATE

Type of pickle	Analytical procedure	
	Colorimetric	Gasometric
Pump	2.73	2.73
Cover	1.82	1.93
Spent	1 08	1.15

DETERMINATION OF CHLORIDE

Cured Meat

The chloride content of a suitable portion of the extract may be conveniently determined with high reproducibility and 4 to 5% accuracy by direct titration with 0.1 N silver nitrate, using 1 ml. of a 5% solution of potassium chromate (8, p. 507) as indicator.

The results of 25 duplicate determinations, picked at random from a large number made on Wiltshire bacon containing from 1.8 to 6.0% sodium chloride, showed an average variation of $\pm 0.15\%$ from their means.

A measure of the accuracy of the extraction procedure, and the direct titration method for determination of chloride in the extract were obtained by comparison with the standard procedure of the A.O.A.C. (8, p. 254). Five different samples of Wiltshire bacon were each analyzed in duplicate by the following three methods:

I. The A.O.A.C. standard method: ignition of the sample followed by Volhard's determination (proteins may be eliminated by wet oxidation also (6)).

II. Twenty-five ml. of the extract treated as in I.

III. Direct titration.

The results appear in Table V, together with certain statistical quantities calculated to determine the significance of the observed differences. Although the standard error of duplicates suggests that the direct titration method is the most precise, the observed differences in these errors by the different methods are not statistically significant. However, the differences between the means by the three methods are significantly greater than their standard errors. Comparison of the results obtained with methods I and II show that the extraction phase of II is satisfactory. The higher values obtained with II may be the result of an error introduced by the impenetrable portion of the meat and its water of hydration when the extract is made up to volume. No correction has been made for this in the results presented here. On the

average the direct titration procedure gives results 4.4% too high, probably because of the presence of protein in the extract.

TABLE V

COMPARISON OF THE EXTRACTION PROCEDURE WITH THE A.O.A.C. STANDARD METHOD FOR THE DETERMINATION OF THE CHLORIDE CONTENT OF CURED MEAT

	NaCl, %		
Procedure	I	II	III
Experimental details	Direct ignition of meat; Volhard titration	Extract of meat evaporated and ignited; Volhard titration	Extract of meat directly titrated by Mohr's procedure
1	6.165	6 330	6 565
2	4 540	4 645	4 855
3	3 355	3 490	3.705
4	3 960	4 065	4 250
5	4 135	4 265	4.415
Mean	4 431	4 559	4.758
Standard error of duplicates	0 0140	0 0125	0 0100

Curing Pickle

The chloride content of any of the three types of pickle can be determined with good reproducibility and accuracy by means of the previously described direct titration procedure on a suitably diluted portion of the sample. As an independent investigation had indicated that the amount of protein commonly present in curing pickle has little effect on the Volhard method for chloride, three pickles were analyzed by both this and the direct titration procedure in order to determine the accuracy obtainable with the latter. The results, shown in Table VI, indicate close agreement between the two methods. The reproducibility is also satisfactory, since the values for 75 duplicate analyses had an average variation of ±0.02% about the mean.

TABLE VI

COMPARISON OF DIRECT TITRATION AND VOLHARD PROCEDURES FOR THE DETERMINATION OF SODIUM CHLORIDE IN CURING PICKLE

Pickle sample	NaCl, %		Deviation of direct titration from Volhard, %
	Direct titration	Volhard	
1	29 1	28 9	+0 69
2	31.2	31.0	+0 65
3	30 9	31 0	+0 32

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Effectiveness of Benzoic Acid Ice for Fish Preservation*

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ABSTRACT

Only a very slight improvement in keeping quality of dressed halibut (*Hippoglossus hippoglossus*) and black cod (*Anoplopoma fimbria*), as evidenced by differences in viable bacterial population and trimethylamine content of excised muscle, results when the fish are stored in crushed tap water ice containing 0.1 per cent benzoic acid instead of in similar ice without this compound. The methods employed for a relatively simple determination of both viable bacterial population and trimethylamine content of the same sample of muscle are described, and the limitations of these as criteria of the relative age of dressed fish stowed in crushed ice are discussed. The "tyrosine" reaction failed to show greater effectiveness for benzoic acid ice; the values increased during storage, but were too irregular to serve as a safe criterion of spoilage.

A.   Evidence from Bacteria and Trimethylamine

By H. L. A. TARR

The results of experiments in which sodium hypochlorite, carbon dioxide, sodium chloride and ozone have been used in attempts to improve the keeping quality of round fish or fillets have been recorded from time to time (Bedford 1932; Chen and Fellers 1926; Coyne 1933; Genin 1936; Hurequin 1936; Killeffer 1930, and Stansby and Griffiths 1935). In a few cases certain of these compounds have been incorporated in the ice employed for icing round fish. Moran and Pique (1925); Gibbs (1923); Chen and Fellers (1926) and Hurequin (1936) reported improvements in the keeping quality of fish iced with hypochlorite ice, while Hurequin (1936), Bedford (1938 and unpublished) and Brocklesby and Riddell (1937) observed similar slightly beneficial effects when using ozonized ice. In 1936 Bedford, working at this Station, suggested that benzoic acid might enhance the keeping quality of halibut stored in ice in which one tenth of one per cent of this compound was incorporated. Preliminary experiments were carried out in which halibut were iced with ordinary tap water ice and with similar ice containing one tenth of one per cent benzoic acid, and the results indicated that the fish kept in the antiseptic ice appeared to retain their freshness somewhat longer as judged by decreases in viable bacterial population and trimethylamine content of the muscle (Bedford 1936 and 1938; Brocklesby and Riddell 1937). However, the results of this work were not conclusive enough to permit of any

327

final decision being made regarding the possible commercial value of such an ice, and the experiments herein described were undertaken with the purpose of obtaining more data regarding its antiseptic qualities. Only bacterial counts and trimethylamine determinations have been used as criteria of the relative keeping quality of the fish in these experiments, since organoleptic tests, though admittedly a very valuable index of quality, must be conducted by a fairly large number of individuals if consistent results are to be expected, as the work of Young (1938) has shown.

EXPERIMENTAL

METHODS OF SAMPLING FISH

Halibut weighing from about 5 to 20 lb. (2 to 9 kg.) and black cod from about 2 to 7 lb. (1 to 3 kg.) were used. The conditions of capture, transport and storage of the fish varied somewhat and are therefore described briefly in each experiment. Sampling a whole fish for the purpose of determining its freshness presents many difficulties, for it is probable that the bacteriological and biochemical changes in different parts of the muscle will not take place at a uniform rate. Thus the bacteria tend to be localized near the cut surfaces of the fish at first and later to penetrate to more remote portions (Harrison et al. 1926). If bacterial counts are to be employed as one criterion of freshness, any attempt to sample the whole muscle of the fish by filleting it under aseptic conditions presents great difficulties, for not only is it almost impossible to prevent external contamination, but also the labour involved would make such a procedure impracticable. The logical solution of the problem appears to lie in sampling approximately the same area of muscle in each fish, with the anticipation that the changes which take place there will be fairly constant for fish which have been stored under given conditions for the same length of time, and will therefore provide some estimate of the age and condition of the fish, at least for comparative purposes. Bedford (1937b) suggested that the bacterial content of the "poke" muscle of halibut might be employed as an index of the quality of the fish, because this region would naturally tend to be most heavily infected and poor methods of handling the fish, such as faulty dressing, poke washing and icing would all contribute to a rapid increase in the bacteria in this area. In the experiments to be described both "poke" and "belly" muscle of halibut have been sampled, and the actual region from which the muscle was excised is recorded in the individual experiments (*vide infra*).

DETERMINATION OF VIABLE BACTERIA AND TRIMETHYLAMINE

In order to obtain muscle under relatively aseptic conditions the slime is first scraped off and the skin of the fish is then swabbed thoroughly with absorbent cotton moistened with 1 per cent mercuric chloride solution. After a few minutes an area of skin somewhat greater than that from which it is desired to excise the muscle is removed under aseptic conditions, about 20 to 50 g. of the exposed muscle is transferred to a sterile petri dish and is minced fairly finely with sterile scissors. 22.5 ml. of sterile water are pipetted into a sterile tube with a graduation mark at 30 ml. and the minced muscle is selected at random and added to the

water until the liquid reaches this mark, thus making a suspension of muscle equivalent to approximately 1 g. in every 4 ml. The aqueous suspension of minced muscle is transferred to a specially made pyrex tube 2.5 cm. in diameter and about 18 to 20 cm. long, which contains 15 g. of fairly coarse washed sand (Baker's quartz sand is suitable), has a stirring rod passing through a rubber stopper and has previously been sterilized. This type of grinding tube has been used by Haines (1937) for determining the bacterial content of samples of meat. The suspended muscle is then ground rapidly, for 1 minute only, using a small electric motor, since longer periods of grinding do not appear to yield higher viable bacterial counts and very prolonged grinding periods (10 to 40 minutes) usually cause a definite decrease in bacterial numbers. Evidently the bacteria occur in the muscle fluids and are fairly easily suspended by brief grinding. This procedure has the distinct advantage of avoiding tedious filtration, for the aqueous muscle extract can be readily pipetted through the sand, leaving the coarse debris behind. The dilute extract thus obtained is employed for determination of viable bacterial counts and trimethylamine as follows.

BACTERIAL COUNTS

After some preliminary comparative experiments the "roll tube" method of Wilson (1922) was adopted for determining the viable bacterial population, since it yielded similar, and, in many instances, slightly higher counts than the more tedious and expensive "plating" method. Duplicate 1-ml. portions of the muscle juice (equivalent to 0.25 g. of muscle), or of suitable dilutions prepared from it, are added to 2-ml. portions of molten (45°C.) Bacto nutrient agar (prepared double strength) in 15 by 1.5 cm. tubes. These tubes are rolled, then incubated for 5 days at 25°C., and the colonies which develop counted. A few experiments with agar media prepared from halibut muscle autolysed for 24 hours at 37°C. over chloroform, or with tryptic digests of this tissue prepared according to the method employed by Hartley (1922) for meat digests, have indicated that these nutrient substrates rarely yield higher viable counts than does ordinary nutrient agar, probably because the bacteria normally associated with fish spoilage are not particularly fastidious with respect to their nutrient requirements.

TRIMETHYLAMINE

The method of Conway and Byrne (1933) as modified by Beatty and Gibbons (1937) for the micro-estimation of trimethylamine was followed in general. With laboratory made dishes somewhat larger than the ordinary "Conway dish", 4 ml. of the dilute muscle juice (equivalent to 1 gram of muscle), 0.5 ml. of approximately 36 per cent formaldehyde, 1 ml. of saturated potassium carbonate solution, and an overnight incubation period of 16 to 24 hours at 25°C. gave very satisfactory results and close duplicates. Freshly prepared 0.01N acid and alkali solutions were used for neutralizing the distilled trimethylamine and for back titrating the excess acid respectively.

In all the experiments the gutting, washing and icing of the fish was either done by, or under the supervision of, the writer, in order to ensure that all procedures were carried out correctly both in the fishing vessel and on shore.

EXPERIMENT 1

Twenty-eight halibut were dressed and the pokes scraped and washed with sea water as soon after catching as possible. One half the fish, selected at random, were iced with ordinary crushed tap water ice and the other half with similar ice containing 0.1 per cent benzoic acid, the fish being stowed in the boat's hold. After about 24 hours they were unloaded, and after being re-iced with their respective ices in large boxes were stored in the ice room of a local fish company. The boxes were placed on large blocks of ice in order to help prevent too rapid melting of the ice surrounding the fish, and these were re-iced regularly throughout the 20 days of the experiment with the two ices. Individual fish were withdrawn at intervals for determining the degree of spoilage, and each fish was sampled in two regions as follows. Poke muscle was sampled by excising a representative portion of the muscle covering the ventral (white side) abdominal cavity region, carefully avoiding penetrating the poke itself, which is lined only by a thin membrane. Belly muscle was obtained by excising muscle from an area on the ventral side approximately midway along the dorsal fin and between this fin and the median line, but not penetrating quite to the bones. The results of this experiment are given in table I, the figures representing averages of duplicate determinations in each case.

TABLE I. Viable bacterial population and trimethylamine content of poke and belly muscle of dressed halibut iced with 0.1 per cent benzoic acid ice and with tap water ice.

Age of fish in days after catching	Estimation of the degree of spoilage in poke muscle				Estimation of the degree of spoilage in belly muscle			
	Ordinary ice		Benzoic acid ice		Ordinary ice		Benzoic acid ice	
	*Bacterial counts	*Trimethylamine	Bacterial counts	Trimethylamine	Bacterial counts	Trimethylamine	Bacterial counts	Trimethylamine
1	195	0.07	106	0.02	0	0.09	0	0.00
2	12	0.05	96	0.16	2	0.02	2	0.07
3	241	0.16	15	0.00	0	0.09	0	0.00
4	3,082	0.05	114	0.16	26	0.00	2	0.10
6	230	0.18	2,180	0.06	1	0.11	0	0.04
8	425	0.11	684	0.13	4	0.05	0	0.04
9	1,660	0.23	980	0.13	–	– —	–	– —
11	117,200	1.87	22,520	0.02	24	0.27	0	0.00
13	1,704,000	1.53	243,400	0.20	66	0.27	20	0.14
14	484,000	2.12	1,440,000	0.14	40	0.33	4,100	0.13
15	24,800	7.54	15,740,000	0.44	—	– —	—	– —
17(a)	13,420,000	7.00	1,340,000	0.39	228	1.89	124	0.11
17(b)	19,160,000	3.52	9,620,000	1.73	—	– —	–	– —
20	172,000	16.16	350,000	0.11	105,400	4.40	8	0.21

*Note—In this and subsequent tables the viable organisms are given as number of colonies per gram of wet fish muscle and the trimethylamine as milligrams $(CH_3)_3N$ nitrogen per 100 g. of muscle.

EXPERIMENT 2

Thirty-two halibut were iced with ordinary ice in the round on board the fishing boat shortly after catching. After about 18 to 20 hours they were landed, dressed, and beheaded and the pokes scraped and washed with tap water. The fish were beheaded in order to simulate commercial shipping practice as the area of muscle liable to infection is naturally increased thereby. One half the fish were iced with 0.1 per cent benzoic acid ice, the remainder wih ordinary ice, and stored as in experiment 1. They were only sampled in one region in this experiment, namely between the dorsal fin and the posterior part of the curve in the median line on the white surface, and the results are recorded in table II.

TABLE II. Viable bacterial population and trimethylamine content of belly muscle of dressed and beheaded halibut iced with 0.1 per cent benzoic acid ice and with tap water ice.

Age of fish in days after catching	Degree of spoilage in ordinary ice		Degree of spoilage in benzoic acid ice	
	Bacterial counts	Trimethylamine	Bacterial counts	Trimethylamine
2	1	0.14	0	0.13
5	0	0.11	1	0.04
6	1	0.13	3	0.13
7	3	0.07	0	0.08
9	0	0.07	0	0.06
12	330	0.31	8	0.17
13	11	0.10	8	0.00
15	108	0.24	4	0.24
16	1,552	0.29	28	0.16
19	80	0.61	36	0.13
20(a)	6,160	0.75	28	0.51
20(b)	2,120	2.75	114	0.33
21	5,400	7 60	1,460	0.45
23(a)	360	4 21	1,900	0.41
23(b)	12,060	5.44	60	0.30
24	4,160	13.90	580	0.46

EXPERIMENT 3

Black cod which had been iced in the round aboard the fishing boat for about 2 days were used. They were dressed, beheaded, the pokes scraped and washed with tap water and iced, 11 of them with ordinary ice and the same number with 0.1 per cent benzoic acid ice. The fish were stored and re-iced with their respective ices as in experiment 1. The sample of muscle for analysis was taken on one side of the fish in the region between the middle of the first and second dorsal fins and from these fins down to practically the edge of the poke, care being taken not to go as deep as the bones nor to penetrate the poke skin itself. In table III the experimental results are given.

TABLE III. Viable bacterial population and trimethylamine content of black cod iced with 0.1 per cent benzoic acid ice and with ordinary ice.

Age of fish in days after catching	Degree of spoilage in ordinary ice		Degree of spoilage in benzoic acid ice	
	Bacterial counts	Trimethylamine	Bacterial counts	Trimethylamine
4	24	0 27	6	0 44
6	50	0.45	8	0.37
9	2,240	0.33	4,100	0.21
11	1,940	0.27	1,820	0.28
13	13,600	0 35	9,600	0.30
14	15,000	0 49	620	0 27
16	56,000	0.50	26,200	0 30
18	76,800	0.69	50,600	0.28
20	8,800	4 75	4,000	0.33
23	80,000	5.62	70,400	2.62
25	9,200	2 44	26,800	0.62

DISCUSSION

It is apparent from the results obtained in the three carefully controlled experiments described that the keeping quality of halibut and black cod is only slightly enhanced, as judged by the bacterial and trimethylamine content of the excised muscle, as a result of storing them in ice containing 0.1 per cent benzoic acid instead of in ordinary tap water ice. Moreover this slight improvement is only evidenced after 9 to 14 days in the ice, depending on which region of the fish is sampled. Fish iced with benzoic acid ice usually, but not invariably, have slightly lower bacterial counts, and a more markedly lower trimethylamine content, than do similar fish iced in ordinary ice. This definite suppression of trimethylamine formation in the presence of benzoic acid has been observed by Brocklesby and Riddell (1937) in the case of halibut stored in ice, and by Tarr and Sunderland (1938) in fillets treated with benzoic acid containing brines. As yet the reason for this is not known, though, in view of the results obtained during a study of the formation of trimethylamine by certain bacteria associated with spoiling fish muscle, it may be due to a suppression of the organisms which reduce trimethylamine oxide to trimethylamine (Tarr 1938, 1939).

These results are not entirely unexpected for it is well-known that benzoic acid is a relatively weak antiseptic, especially in the form of its neutral salts such as are probably formed when the aqueous solution of the free acid from the melting ice comes in contact with the buffering substances present in the slime and other parts of the fish. However, it must not be forgotten that benzoic acid ice is practically a sterile ice bacteriologically (Bedford 1938) even if it has only weak sterilizing properties, and as such may yet prove to be an essential link in the chain of clean fish handling methods, especially in cases where ice is manufactured from water of high bacterial content. In this connection it must be recalled that fresh water ice has been shown to be at least one source of *Pseudomonas*

fluorescens, an organism which has been found to be associated with, and is capable of causing, the yellow discolouration of halibut (Harrison and Sadler 1929). A possible objection to the use of benzoic acid in ice lies in the fact that this compound is at present prohibited in fish and fish products (Food and Drugs Act, Ottawa, 1938).

The bacterial counts of the ventral poke muscle of iced halibut are consistently very much higher than those of the belly muscle of the same fish, and this is true of the trimethylamine content, especially in the case of fish iced with ordinary ice. These results might be expected from the fact that the bacteria causing decomposition find access through the cut surfaces near the belly cavity and gills, and initiate putrefaction in the nearby muscle. They are not, however, in agreement with those of Brocklesby and Riddell (1938), who found no significant differences in the trimethylamine content in different regions of iced halibut studied over a period of fifteen days.

Some interesting information regarding the probable value of the trimethylamine test and viable bacterial counts as criteria of the age of fish stored in ice has emerged from these results. Bedford (1938) suggested that the poke muscle would be the most sensitive region to sample in order to obtain an estimate of the condition of halibut. This is to some extent true, for very much higher viable bacterial counts and trimethylamine values are obtained in this region, and also deterioration commences sooner here. However, the bacterial counts and trimethylamine content of this muscle may be practically identical in two different fish even when one of them has been stored in ice four or five days longer than the other. This is probably due to the fact that each fish is contaminated to a different degree, and that in some the thin skin lining the poke is more severely lacerated than in others, thus facilitating the entry of microorganisms in greater numbers. Similar irregularities are evidenced in the case of the belly muscle of halibut and of black cod, and it would therefore appear that the use of viable bacterial counts or trimethylamine determinations as indices of the age of fish stored in ice can only yield very approximate results. The use of the trimethylamine determination alone as a criterion of freshness must be considered with particular caution since it has been shown that only certain types of bacteria form trimethylamine (Tarr 1938, 1939) and the proportion of these in spoiling fish muscle may be extremely variable. Before attempting to apply any single test as a sole index of the freshness of round fish it will not only be necessary to determine the relative roles of autolysis, direct bacterial action and the diffusion of the products of bacterial metabolism from the more highly infected regions to less highly contaminated ones, but also to carefully correlate organoleptic tests, viable bacterial counts and trimethylamine values in fish of different age.

icing of the fish and to McCaffery's Cold Storage for freezing and crushing the ice used. The permission granted by Mr. J. Dybhavn to store the fish in the ice room of the Royal Fish Company is greatly appreciated.

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B. Evidence from the Tyrosine Reaction

BY B. E. BAILEY

The blue colour given by tyrosine with the molybdic phosphoric reagent of Folin and Denis (1912) and Folin and Ciocalteu (1927) can be applied as a sensitive method for detecting the primary breakdown of proteins. This reaction was used by Bradley (1922) to measure autolysis. Its correlation with the primary cleavages in proteolysis was studied by Torbet and Bradley (1931). Anson (1938) has used it in his method for the estimation of pepsin, trypsin and cathepsin. In this paper it is called the tyrosine reaction merely for convenience. Actually it measures the reactive tyrosine complexes and other phenols, tryptophane, cystine, sulphhydryl compounds and other reducing substances. The reagent is thus particularly applicable to the detection of tissue spoilage, since a number of the compounds which develop in the spoilage process will give the colour.

This reaction was applied in the present work to extracts of the muscle of halibut stored in benzoic acid ice and in ordinary ice. This was done for two reasons. In the first place it was thought that this additional measure of the

change in the fish muscle would aid in assessing the value of the benzoic acid ice as a preservative, and secondly it was desired to compare the tyrosine reaction with the trimethylamine content as an indicator of spoilage. It was applied to the halibut of experiments 1 and 2 (pages 330 and 331), the samples being taken in similar regions to those for bacteria and trimethylamine, but on the dorsal side of the fish.

TABLE IV. Tyrosine reaction (mg. tyrosine per g. of muscle) of muscle of halibut iced with 0.1 per cent benzoic acid ice and with tap water ice

Days after catching	Ordinary ice		Benzoic acid ice	
	Poke muscle	Back muscle	Poke muscle	Back muscle
1	0.087	0.087	0.091	0.089
2	0.098	0.096	0 098	0.090
3	0 108	0.093	0.112	0.098
4	0.125	0.106	0 127	0.109
6	0.112	0.112	0.091	0 095
8	0 131	0 120	0 128	0 106
9	0 229	0.167	0.181	0.156
11	0 118	0 104	0 180	0.164
13	0.224	0.133	0.161	0 139
14	0.146	0.122	0 190	0 139
15	0.317	0 171	0.227	0 180
17(a)	0 325	0 165	0.220	0 215
17(b)	0 265	0 174	0 192	0 198
20	0 326	0.187	0 407	0 199

TABLE V. Tyrosine reaction (mg. tyrosine per g. of muscle) of back muscle of halibut iced with tap water ice and with 0.1 per cent benzoic acid ice

Days after catching	Ordinary ice	Benzoic acid ice
2	0.090	0 093
6	0 123	0 132
7	0 151	0 139
9	0 234	0 200
12	0.174	0.151
13	0.183	0 181
15	0 214	0 238
16	0.184	0 197
19	0.127	0 202
20(a)	0 230	0.218
20(b)	0.301	0 266
23(a)	0.294	0 296
23(b)	0 305	0.250
24	0.452	0.356

PREPARATION OF SAMPLES

The samples were prepared for the tyrosine determinations as follows. A small piece of muscle was finely minced and mixed thoroughly. Twenty grams of the minced muscle were weighed out in a small Erlenmeyer flask and 80 ml. of water added. The flask was shaken at frequent intervals for 30 minutes. A 10 ml. aliquot of the muscle suspension was then mixed with 10 ml. of water and 20 ml. of ten per cent trichloracetic acid. After standing for at least two hours the mixture was filtered, and the tyrosine reaction of the filtrate determined. The results are given in tables IV and V.

DISCUSSION

While the tyrosine values increased from the beginning of the experiment, they were irregular, due to considerable variation between the values given by different individual fish. Higher values were found for the muscle adjacent to the poke than for the back muscle after a week in storage. The tyrosine reaction did not indicate that there is any appreciable advantage to be gained from storing the fish in benzoic acid ice, the values obtained being as great, in general, in fish stored in this ice as in ordinary ice.

By comparing the tyrosine values with the trimethylamine content of the same samples (pp. 330, 331), it can be seen that the latter gave a better measure of the relative freshness than did the tyrosine reaction. The trimethylamine content remained very low until the fish had begun to spoil, when a rapid increase occurred. The tyrosine values, on the other hand, showed such a marked variation between individual halibut that it would be difficult to establish a level above which it might safely be predicted that the fish would be unfit for use as food.

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SURFACE DRYING OF FROZEN POULTRY DURING STORAGE [1]

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If market poultry is to be stored for periods of over a month it must be frozen in order to avoid spoilage from microbial activity, Lochhead and Landerkin (1935). In ordinary frozen storage one of the first forms of serious deterioration that occurs is the development of lighter-colored circular spots around the feather follicles and discolored areas of irregular shape on the skin surface. These markings are commonly referred to as "pock-marking" and "freezer-burn," respectively.

The results of Tressler (1935) and Moran and Wright (1937) have shown that surface drying is the primary cause of these defects. There is, however, little quantitative information available concerning the rate of drying at the temperatures and relative humidities commonly used for frozen storage. This investigation was undertaken to secure such information, and also to determine the value of different methods of packaging for preventing deterioration from surface drying.

FACTORS AFFECTING RATE OF DRYING

Although the principal factors determining the rate of drying are comparatively well understood, it appeared worth while to consolidate such of this information as is relevant to the drying of frozen products at this point. Briefly, the rate of drying of any product depends on two sets of conditions: first, the temperature, relative humidity, and movement of the surrounding atmosphere; and second, the rate of movement of moisture from the interior of the product to the evaporating surface. Obviously surface desiccation will occur whenever the removal of moisture from the surface is more rapid than its movement from the interior of the product to the surface.

Since most of the moisture in frozen products is in the solid state, its rate of movement to the surface is reduced to negligible proportions. Under these conditions surface drying can occur, and although the over-all loss of weight may be small, it may have a

serious effect on the appearance. In chilled (unfrozen) products the internal movement of moisture is not so seriously restricted, and greater over-all losses in weight can occur with less serious effects on the surface appearance. Obviously the surface desiccation of frozen products can be prevented only by reducing the rate of evaporation from the surface.

The drying of wet materials has been reviewed by Fisher (1935), who shows that the rate of evaporation is given by:

$$\frac{dw}{dt} = k(p_w - p_a)$$

where $\frac{dw}{dt}$ is the rate of evaporation; k a constant dependent in this case on the velocity and flow characteristics of the air; and p_w and p_a the vapor pressure of the evaporating water and that of the air, respectively. This equation has been found to apply under a wide variety of conditions, but it is not known whether it is strictly applicable to evaporation from a frozen product where most of the water is in the solid state. Since the quantity $(p_w - p_a)$ appears in most drying equations, however, it seems reasonably certain that the rate of evaporation from a frozen product is a function of this quantity.

Since the vapor pressure of water (p_w) and the moisture-holding capacity of the air (p_a) both decrease as the temperature is lowered, it is evident that the quantity, $p_w - p_a$, and the rate of drying will generally decrease as the temperature is lowered. At constant temperature p_w is fixed, but p_a will vary with the relative humidity. Thus the rate of drying can be reduced by lowering the temperature, increasing the relative humidity, or by a combination of these changes.

Moran (1934) places the eutectic temperature of the liquids in muscle in the region of —37.5°C.(—36°F.); and since most products start to freeze at about —1°C.(30.2°F.), it follows that at temperatures between these extremes, such as are normally used for frozen storage, the majority of the water will be present as pure ice or have an equivalent vapor pressure. It appears, therefore, that the vapor pressure of ice may be taken as a satisfactory estimate of p_w.

The value of p_a to be used in the drying equation is difficult to establish at freezing temperatures. Although ice is the stable state, Keyes and Smith (1934) point out that air appears to exert a solvent action, which becomes proportionately large at low temperatures, and retains a quantity of water vapor more closely related to the vapor pressure of supercooled water than to that of ice. Relative

humidity is defined in terms of the moisture contained in the air and not on the vapor tension. Since most metereological tables report relative humidity as usually defined, Ewell (1934), they represent the practical equivalent of vapor tension relative to that of super-cooled water. Atmospheric vapor tensions computed on this basis could then exceed the vapor pressure of ice, and the drying equation would indicate condensation rather than evaporation at high relative humidities. For this reason it seemed preferable, at freezing temperatures, to express the vapor tension of the atmosphere as a percentage of the vapor pressure of ice at the same temperature. This quantity may be termed "humidity relative to ice," and at frozen storage temperatures this quantity may be 15 to 20 per cent higher than the corresponding relative humidities as ordinarily de-fined and computed.

EFFECT OF STORAGE TEMPERATURE AND HUMIDITY

In preliminary experiments frozen poultry was stored in closed containers over ice at temperatures of —13.5°C.(7.5°F.) and —22°C. (—7.5°F.) for periods of over a year. No evidence of surface mark-ing appeared on any of these birds. This showed that freezer-burn and pock-marking were caused by surface drying, since it did not occur in a space having a vapor pressure equal to that of ice. Simi-lar birds exposed in storage rooms at the same temperature but about 70 per cent relative humidity showed evidence of pock-mark-ing, followed by freezer-burn, within two or three months. Since storage periods in excess of two or three months are usually desired, these results indicated that a study of the rate of drying at humidi-ties between 70 and 100 per cent relative to the ice was of primary interest.

Temperatures of —13.5°C. and —22°C. were again chosen for the main series of experiments, since these are representative of the upper and lower part of the temperature range commonly used for storing poultry. These temperatures were maintained well within ±.5°C. throughout the greater part of the storage period. Occa-sional deviations of greater magnitude were of short duration.

Ice or calcium chloride solutions of appropriate concentration were used to obtain the desired humidities. The vapor pressure of calcium chloride solutions at sub-zero temperatures has been com-puted by Linge (1929) from known physical constants and thermo-dynamic considerations, while Awbery and Griffiths (1937) have reported experimental results. When the vapor pressures reported by these independent investigators were computed to humidities relative to ice, the two sets of values were in good agreement for

high humidities at temperatures just below the freezing point. As the humidity and temperature decreased, however, the values became divergent, the experimental results being as much as five per cent higher than the calculated values at a humidity of 85 per cent at —20°C.(—4°F.). On the basis of humidity relative to ice, both sets of data show that this quantity increases, for a solution of given concentration, as the temperature is lowered and naturally reaches 100 per cent at its freezing point. This reference point was therefore used for making the necessary extrapolations to obtain the vapor pressure of the solutions at the lower storage temperature, —22°C. These extrapolations could be made with greater assurance from Linge's data than from the experimental results. In consequence, all humidities reported subsequently were computed from Linge's values.

The initial concentration of the solutions was adjusted to give the following humidities relative to ice, at both storage temperatures: saturated solution (humidity unknown) 75, 80, 85, 90, 95, and 100 per cent (ice). The exact humidity of the atmosphere surrounding the product during the 83-week storage period was, however, subject to uncertainty arising from the following sources: (1) the divergence between calculated and experimentally determined vapor pressures as mentioned above; (2) dilution of these solutions with consequent increase in humidity during storage; and (3) the actual relative humidity of the air in the container which doubtless assumed some intermediate value between that corresponding to the vapor pressure of the product (ice) and the solution. During the storage period attempts were made to measure the relative humidity of the air in these somewhat small closed containers, but all of these failed to give sufficiently accurate results. Toward the end of the storage period a special hair hygrometer, calibrated over solutions of calcium chloride of known concentration and over ice, appeared to give reasonably reliable values at the higher storage temperature. At the lower temperature, —22°C., it was not sufficiently sensitive to give accurate readings.

In view of these considerations the reported humidities must be regarded as relative rather than absolute values. Nevertheless, it seemed desirable to form some estimate of the mean effective humidity to which the product was exposed. Although it was impossible to measure the concentration of the solutions during storage, this was done at the end of the experiment, and the corresponding humidity, relative to ice, computed. Although the rate of dilution doubtless decreased throughout the storage period, the total dilution was usually small, and the effective humidity was consequently taken as

the arithmetic mean of the initial and final humidities. Since the evaporating surfaces of the solution and product were of comparable magnitude, the relative humidity of the air was computed from the average of the mean vapor pressure for the solution, as discussed above, and for the product (ice). The only justification for making these computations is that they probably indicate the mean effective humidity more closely than either the initial or final humidities over the solutions. Actually the magnitude of both these corrections, and the uncertainty of the exact vapor pressure of the solutions decreases as the humidity increases. At calculated relative humidities of about 95 per cent or higher these are probably close estimates of the absolute value. Nevertheless, the relative nature of the observations must be recognized.

Three criteria were used for assessing the effect of the storage conditions on the product: (1) the time required for definite evidence of surface drying; (2) the percentage of the surface area affected at the end of the storage period; and (3) the moisture content of the skin. Since the containers were large glass desiccators, the product could be examined periodically without opening the vessels in order to determine the time required for surface marking to appear. At the end of the storage period the proportion of the skin area affected was estimated. The skin was removed while the birds were still frozen, and then ground and weighed at freezing temperatures for the moisture determinations. The moisture content was determined by drying *in vacuo* at 100°C.(212°F.) to constant weight. Two birds were stored under each set of conditions, and duplicate or triplicate measurements made on each bird to obtain the reported values.

RESULTS OF TEMPERATURE AND HUMIDITY EXPERIMENTS

The summarized results of this series of experiments (Table 1) give the storage temperature, the computed mean effective relative humidity throughout the storage period, and the observed relative humidity, measured with the special hair hygrometer, at the end of the storage period. These observed values are generally higher than the computed humidities. This is to be expected, since the maximum humidity would occur at the end of the storage period and could be higher than the mean effective humidity throughout the period.

The two birds used in each test seldom showed evidence of surface marking at the same time, nor were the proportions of the skin areas affected or moisture contents of the skins the same for the two birds at the end of the storage period. In order to give some

idea of the variability to be expected between different birds stored under the same conditions, the two individual values, rather than their mean, have been reported (Table 1).

The results indicate that at computed mean effective humidities of 95 per cent or lower, the product will show definite evidence of surface marking after two or three months' storage at both temperatures. Detrimental drying did not occur during 83 weeks' storage in a saturated atmosphere at —13.5°C.(7.5°F.) or at humidities

TABLE 1

Effect of Storage Temperature and Humidity on Surface Drying

Storage conditions			Time required for definite evidence of surface drying	Estimated proportion of skin area affected after 83 weeks' storage	Moisture content of skin
Temp.	Humidity relative to ice				
	Computed	Observed			
°O.	pct.	pct.	wk.	pct.	
—13.5	<82	93	8	40-60	21.4-21.8
	90	95	8-10	10-20	35.2-44.0
	92	96	8-14	20-25	32.2-33.4
	94	96	9-10	15-25	29.6-35.6
	96	97	22-24	5-10	42.8-46.8
	98	97	37	Slight	37.1-38.6
	100	100	>83	None	47.7-50.2
—22.0	<76	98	12-14	5-10	52.1-53.2
	89	99	8-9	5-10	43.0-48.6
	91	96	9-12	5-10	39.1-52.2
	93	98	22-26	5-10	48.4-50.1
	96	100 [1]	32-37	0-5	41.3-50.6
	98	100	>83	None	44.4-55.0
	100	100	>83	None	51.1-60.0

[1] Temperature variable when observations were made.

higher than 98 per cent at —22°C.(—7.5°F.). Humidities between about 95 and 98 per cent at —13.5°C. gave safe storage periods of six to nine months' duration. Conditions which caused surface drying to become evident in a relatively short time also caused severe desiccation, affecting a large proportion of the skin area, during long storage periods. At comparable humidities less deterioration occurred at —22°C. than at —13.5°C., a result in qualitative agreement with prediction from the drying equation given earlier.

It was expected that the final moisture content of the skin would constitute the best quantitative estimate of the deterioration from drying. Subsequently it was found that the moisture content of the skin of freshly killed poultry varied considerably, presumably as the result of varying fat content, scince Holcomb and Maw (1934)

have shown that the moisture content of poultry muscle generally decreases as the fat content increases. It therefore seems probable that the final moisture content of the skin was dependent on the initial moisture content (not determined) as well as on the extent of drying under the various storage conditions. Since it was impossible to remove separately and quantitatively the skin from the affected and unaffected areas, the analyses had to be made on the skin from the entire bird. Under certain storage conditions the over-all reduction in moisture content might be small and therefore difficult to distinguish from the initial variations in moisture content.

The results (Table 1) show that the lower values for the moisture content of the skin were generally obtained under the drier storage conditions. Nevertheless, the large differences observed between birds stored under the same conditions made it impossible to draw definite conclusions from inspection. Statistical calculations were therefore made to determine whether the observed differences

TABLE 2

Analysis of Variance of Moisture Content of Skin

Variance	Degrees freedom	Mean square
Between birds stored under same conditions......................	14	11.1548
Between birds stored under different conditions.................	13	85.0626[1]

[1] Indicates one per cent level of significance

between the moisture content of the skin of birds stored under different conditions were significantly greater than the differences between birds stored under the same conditions. The results of such an analysis of variance (Table 2) show that the differences in the moisture content under different storage conditions were highly significant.

When freezer-burn reaches an advanced stage, as it did under certain of these storage conditions, the drying may affect the flesh in certain areas to an appreciable depth. The flesh from such areas, together with a similar piece of apparently unaffected muscle from an adjacent area on the same bird, was removed for moisture determinations. Typical results (Table 3) show that severe freezer-burn may cause a loss of about one-third of the moisture originally present in the muscle.

EFFECT OF METHOD OF PACKAGING

Since the frozen product has a vapor pressure equivalent to that of ice at the same temperature, it is evident that the application of an effective moisture-proof lining material in the package should

allow the air surrounding the product to rise to 100 per cent rela-
tive humidity, and thus prevent freezer-burn. Poultry boxes are
usually lined with some form of paper, and the adjoining edges are
ordinarily overlapped but not sealed. Although relatively moisture-
resistant paper stocks are now commonly used as lining materials, it
is difficult to see how these can maintain the high humidities required
to prevent freezer-burn if left unsealed. Some experiments were
therefore made to determine the value of sealing the lining mate-
rials.

As it was not the object of these tests to determine the relative
value of the many moisture-resistant lining materials available, only

TABLE 3

Moisture Content of Flesh From Affected and Unaffected Areas

Bird No.	Moisture content	
	Affected area	Unaffected area
	pct.	*pct.*
19	52.3	**73.4**
30	54.2	71.2

three paper stocks were used. One of these was parchment, chosen
as typical of the moisture-permeable materials; the second was a
heavy waxed paper, taken as representative of the better type of
moisture-resistant materials; and finally a heavy aluminum foil (.01-
inch) solidly laminated to a white sulphite paper. Since this thick-
ness of aluminum foil is normally quite free from pinholes, this lining
material was considered to be completely impermeable to water vapor.

The boxes with unsealed liners were packed in the usual way,
allowing a generous overlap where the sheets joined. In the boxes
with sealed liners the paper stock was placed in the boxes, and the
joints on the bottom and sides were sealed on the inside with molten
wax before the birds were packed. After packing, the lining material
was folded over the birds and sealed in a similar manner on the
outside. All tests were made on duplicate boxes of poultry.

The hair hygrometer, previously mentioned, was used to deter-
mine the relative humidity inside the liners during the storage period.
In order to accommodate the sensitive part of this instrument, a per-
forated brass tube was inserted in each end of all boxes at the time
of packing. This tube was secured in, and flush with, the end of
the box on the outside. Where it passed through the liner, the latter
was adequately sealed both to the tube and to the end of the box. The
end of the tube was stoppered on the outside when the hygrometer
was removed.

When packed, the poultry was frozen and stored in a room at —13.5°C.(7.5°F.), a temperature at which freezer-burn usually becomes evident in a few weeks. The relative humidity in this room was 69 ± two per cent except for short periods when it may have been somewhat higher. The storage period between packing and the final examination was about a year; but as it was impossible to pack and examine all of these boxes at one time, the storage period for individual boxes varied from 47 to 59 weeks.

The relative humidity in the boxes was observed periodically throughout the storage period. Since the hygrometer usually required 48 hours or more to reach equilibrium at this temperature, as well

TABLE 4

Effect of Method of Packaging

Material used and method of lining	Observations during storage		Final observations		
	Time after freezing for relative humidity to reach 95%	Estimated safe storage life	Number of weeks' storage	Relative humidity	Proportion of area affected
	wk.	*wk.*		*pct.*	*pct*
Parchment, unsealed..............	0-1	8-9	48-57	75-77	55-68
Parchment, sealed........	0-7	8-15	48-59	75-85	45-57
Waxed paper, unsealed..........	1 10	9-18	55 58	95-99	12-22
Waxed paper, sealed.	56+	64+	47-56	98 100	2-12
Aluminum foil on sulphite stock, unsealed......	28 48	36 56	47-53	82 95	8-10
Aluminum foil on sulphite stock, sealed....	48+	56+	47-48	100	0-1

as frequent calibrations, it was only possible to obtain one observation per box per month on the average.

RESULTS OF PACKAGING EXPERIMENTS

In all of the boxes the relative humidity reached about 100 per cent during freezing and then fell off gradually during storage. These results have been summarized (Table 4) by reporting the time required for the humidity to fall to 95 per cent and also the final relative humidity just before the box was opened for examination. Since the results (Table 1) show that surface marking will usually occur after about eight weeks' storage at —13.5°C. at relative humidities below 95 per cent, the safe storage period for each method of lining was estimated by adding eight weeks to the period required for the humidity to reach 95 per cent. This value is also reported (Table 4) together with the number of weeks each box was

stored, the final relative humidity, and the proportion of the skin area affected when the final examination was made. In most cases two values are reported to give some indication of the observed differences between the duplicate boxes.

It is evident from the results that the several criteria used for estimating the quality of the liner—the estimated safe storage life, the final relative humidity, and the proportion of the skin area affected—are generally in good agreement. The results also confirm the findings of the previous experiment by showing that relative humidities of 98 per cent or higher are necessary to reduce freezer-burn to small proportions over a one-year storage period at —13.5°C.

With reference to the three materials used as liners it is evident that freezer-burn was most severe within the moisture-permeable parchment, less within the relatively impermeable waxed paper, and least within the impermeable aluminum foil. This result was to be expected, but it is of interest to note that a practical test of this nature is capable of demonstrating the value of the impermeable aluminum foil over the relatively moisture-proof waxed paper.

These experiments were designed essentially to determine the value of sealing the liner. The results (Table 4) show definitely that sealing all three materials prolonged the safe storage life and reduced the proportion of the skin area affected at the end of the experiment. It is worth noting that sealing improves a moisture-permeable material, such as parchment. Again a sealed, wax-paper liner appears to be equally as good as an unsealed, impermeable liner, such as aluminum foil. This indicates that the full benefit of a relatively impermeable liner can be obtained only if it is sealed. The aluminum foil used in these tests, although desirable, would probably prove too costly in commercial practice. A lighter weight foil, however, might prove to be equally effective and economically feasible. Sealing the relatively, but not completely, impermeable stocks (such as waxed paper) now in common commercial use would improve their utility. The development of a package capable of being easily and effectively sealed in practice would appear to be justified.

SUMMARY

An experiment, involving the storage of poultry for 83 weeks at temperatures of —13.5 and —22°C.(7.5 and —7.5°F.) and several relative humidities, yielded results in qualitative agreement with drying equations obtained with other materials, namely, that the rate of evaporation varies directly with the temperature and inversely with the relative humidity.

The period required for surface desiccation to affect the appearance of the product, the proportion of the skin area affected, and its moisture content after storage, were used as practical estimates of the rate of drying. These criteria showed that humidities less than about 95 per cent at both storage temperatures were unsatisfactory as the product was seriously affected in from two to three months. Humidities of 98 to 100 per cent maintained the product in satisfactory condition, with respect to surface drying, during 83 weeks' storage. Humidities intermediate between these extremes gave safe storage periods of six to nine months' duration. At comparable humidities, surface desiccation was less severe at the lower temperatures. Severely freezer-burned muscle tissue contains only 50 to 55 per cent moisture, whereas normal tissue contains about 72 per cent.

It was found that sealing the joints in the paper liners commonly used in commercial poultry boxes was beneficial regardless of the moisture-permeability of the paper stock used, such as parchment, heavy waxed paper, and heavy aluminum foil. Only the sealed aluminum-foil liner prevented surface marking during storage for one year at —13.5°C. Sealed, moisture-resistant, but not impermeable, stocks, such as heavy waxed paper, were as effective for reducing deterioration from surface drying as an unsealed, impermeable-foil liner.

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Humidification of Freezers[*]

By W. H. Cook[†]

Summary

The atmosphere of a freezer must have a relative humidity of 95 per cent or higher if surface drying of an unprotected product is to be prevented over the ordinary storage periods. Preliminary experiments showed that this condition could be maintained only by the continuous addition of adequate quantities of water vapor to the air under conditions that prevented the formation of ice on the cooling coils. These conditions were met by spraying a sufficiently concentrated liquor over the coils to prevent ice formation, evaporating a portion of the water from this liquor, and returning the resultant steam to the freezer continuously to maintain the desired humidity.

In order to accomplish the maximum evaporation with the minimum heat input it was necessary to heat a portion of the liquor to the boiling point while providing continuous circulation through the heater to prevent troublesome concentration of the non-aqueous phase. Equipment was designed for regulating the proportion of water to be evaporated from the liquor, controlling the rate of flow through the heater, and balancing the heat input with the amount of water to be evaporated.

Experimental tests with this equipment have demonstrated that relative humidities of 95 per cent or higher can be maintained in an experimental freezer. The results also indicated that a linear relation exists between the relative humidity in the space, and the amount of water delivered to the air in unit time. Although this equipment has not been tested in commercial practice, certain deductions made from theoretical considerations and preliminary tests in a small room indicate that humidification of commercial freezers should be feasible economically.

UNDER the conditions prevailing in existing storage spaces maintained at freezing temperatures, surface drying is responsible for the primary forms of deterioration. Surface drying causes two general types of deterioration, a loss of bloom affecting the entire surface,[2] and a more severe desiccation of irregular areas, commonly known as freezer burn. These physical changes primarily affect the appearance and saleability of the product, although it has also been shown[3] that oxidative changes, responsible for the development of rancidity in fats, proceed more rapidly in products that become desiccated at the surface. Deterioration of this sort is commonly reduced or prevented by packaging or wrapping the product in a more or less moisture-proof manner. Surface drying can also be reduced by using very low storage temperatures or by humidifying the freezer at ordinary storage temperatures, and these methods are used to a limited extent in commercial practice. The use of low storage temperatures is undoubtedly effective but frequently uneconomical. Similarly humidification is effective if sufficiently high humidities can be maintained. This paper describes some of the conditions that must be met in the humidification of freezers, the equipment and method used, and certain preliminary results regarding its performance.

The cooling coils in a freezer are ordinarily 10° F. below the air temperature, and in an empty room they condense the moisture out of the air until the dewpoint temperature of the air is the same as that of the coils. This corresponds to about 62 per cent relative humidity theoretically, and actual observations in empty freezers yielded values ranging from 62 to 68 per cent. Frozen perishables, on the other hand, have a vapor pressure similar to that of ice at the same temperature, or the equivalent of 100 per cent relative humidity. When such products are placed in a freezer they lose moisture to the air, and since moisture is being removed continuously by condensation on the coils, the air attains some intermediate humidity between 62 and 100 per cent. The actual value attained depends on a number of factors, but observations in several commercial freezers indicate that it is seldom above 85 per cent when equilibrium is attained, although higher values may prevail temporarily.

Humidity Required and Method of Measurement

BEFORE attempting the humidification of freezers the relative humidity level necessary to prevent economic losses over the usual storage period had to be determined. This information was secured by storing various products at several relative humidities and temperatures. The method employed and the results obtained have been reported elsewhere.[1] Briefly they showed that at a temperature of —13.5°C. (7.5°F.) a relative humidity of about 98 per cent or higher was necessary to prevent visible surface desiccation on an exposed product over a storage period of one year's duration. At a storage temperature of —22°C. (—7.5°F.) somewhat lower humidities were effective for preventing surface drying, a result in qualitative agreement with that predicted by the general form of drying equation.[4] In commercial practice where the storage periods are generally less than one year, and the product is usually protected by some form of covering, it appears that somewhat lower humidities may prove adequate to prevent economic loss. In consequence the maintenance of 95 per cent relative humidity was taken as the first objective.

Since the difference between satisfactory and unsatisfactory storage conditions is determined by a difference of a few per cent of relative humidity in the vicinity of saturation it was necessary to develop a sensitive and precise method for the measurement of relative humidity at freezing temperatures. An extensive study of a number of modifications of several methods led to the use of a modified dewpoint procedure described by Winkler.[5] All of the other methods or procedures tested lacked the necessary precision or sensitivity. Unfortunately the dewpoint method is rather slow and frequently a period of several hours is required to make the necessary observations in duplicate. High-grade hair hygrometers, however, have been found to have the required sensitivity but lack the necessary precision unless they are calibrated frequently at the actual temperature and humidity prevailing in the rooms. By using the dewpoint method to calibrate a suitable hair instrument, a continuous record of the humidity is obtained. Certain of the observations reported in this paper were taken from a hair instrument calibrated daily, or more frequently, by the dewpoint method.

Preliminary Experiments

IN order to humidify a freezer it is necessary to add a continuous supply of water vapor to compensate for the amount removed by the coils in the same period of time. This fact is well recognized and methods of humidification

based on the addition of ice, water or steam are described,[8] or are in use commercially. Preliminary tests of these practices showed that the addition of snow far in excess of the amount that could be tolerated in a commercial freezer failed to raise the humidity to the desired level. When water was introduced into the space the relative humidity attained increased with the water temperature, and the addition of steam raised the relative humidity to 95 per cent, but failed to maintain this level. Following the introduction of steam the humidity increased, and remained at a high level until the coils became sufficiently coated with frost to impair the heat transfer, when the temperature increased and the relative humidity consequently decreased. In the experimental room of about 1125 cu. ft., this condition prevailed after admitting steam continuously for one or two days. Although all of these methods increased the relative humidity, and to that extent reduced the drying power of the air, none of them was capable of maintaining relative humidities of 95 per cent or higher, over extended storage periods.

These results demonstrated that the prevention of ice formation on the cooling surfaces was an essential requirement of any successful method of humidification. This was accomplished in the next series of experiments by circulating a strong brine from a small tank over the cooling coils and returning it to the tank. Since a brine sufficiently strong to prevent the formation of ice on the coils, must have a lower vapor pressure than ice at the same temperature, it follows that no humidification was accomplished by this practice.

Although this brine spray prevented ice formation on the coils it naturally suffered continuous dilution from the absorbed moisture. In order to maintain the desired brine concentration, and accomplish humidification, a small heater was attached to the supply tank which evaporated part of the water, the resultant steam being discharged into the room to maintain the desired relative humidity. This principle forms the basis of the method of humidification to be described. It is possible to employ any suitable freezing point depressant that is not volatile at the boiling point of the liquor, i.e., soluble salts or ethylene glycol, etc. It should be noted that in this system the moisture is removed from the air by the liquor on the surface of the coils, returned to the supply tank, and evaporated into the air by the addition of heat. The loss or gain of moisture from the system as a whole by air exchanges through the door, etc., may increase or decrease the concentration of the liquor slightly, but in practice the concentration seldom requires adjustment more than twice monthly.

If the heater, required for evaporation, is placed in the supply tank, the heat input appears as sensible heat to be removed from the liquor by the cooling coils, and the extent of evaporation or humidification is negligible. By enclosing the heating element in a small section of pipe attached to the supply tank, a small quantity of the liquor can be boiled to effect evaporation and reduce the proportion of the heat input appearing in the sensible form. It is necessary to provide a continuous circulation of the liquor through the heater section in order to prevent troublesome concentration of the non-aqueous phase. At the same time this flow must be restricted to a minimum in order to obtain the maximum evaporation with the minimum heat input.

In the first form of the equipment a continuous slow flow was obtained by using a restricting orifice in the feed line between the main tank and the heated section. With this equipment it was possible to obtain a maximum humidity of about 90 per cent in a 1125 cu. ft. room with an energy input of about 500 watts. The humidity varied over a 5 to 10 per cent range and the apparatus occasion-

ally gave trouble from the choking of the orifice.

In the next form of the equipment a heat exchanger was placed between the hot concentrated liquor returning to the supply tank from the heater and the cold dilute liquor entering the heater from the supply tank. In this apparatus the restricted, but continuous, flow was obtained with full bore pipes by using limited liquid heads to restrict the flow. This equipment was a decided improvement over the earlier form and raised the humidity from 90 to 95 per cent, under the same conditions as those previously described. It suffered from the objection that the heat input and liquid head had to be carefully adjusted. Changes in the density of the liquor resulting from evaporation, and changes in viscosity caused by temperature changes, both affected the rate of flow and consequently the evaporating efficiency for a given heat input.

Apparatus and Method

THESE facts together with information obtained from the application of the equipment to various test conditions, such as variable room temperatures, various temperature differences between the coils and the air, lead to
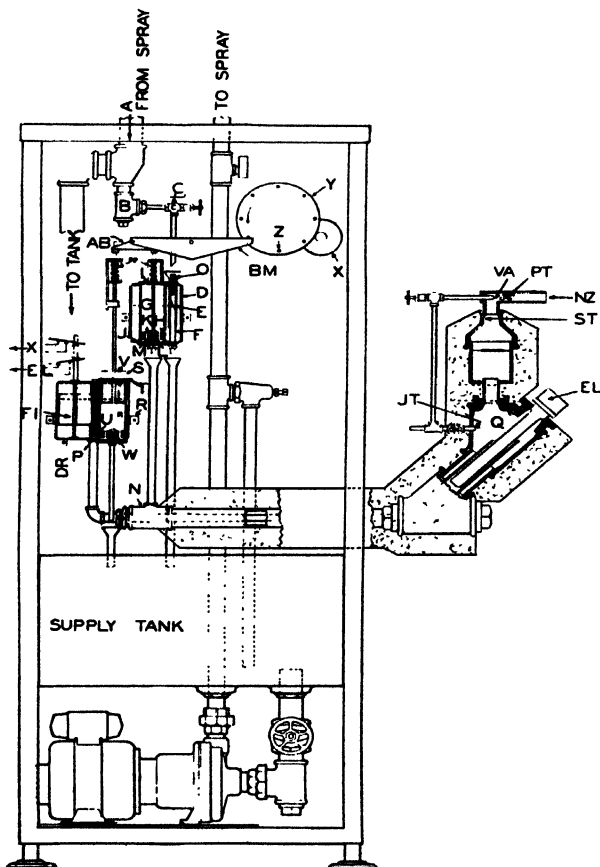
FIG. 1. HUMIDIFYING EQUIPMENT INCLUDING PROPORTIONING AND FLOW CONTROL APPARATUS.

the conclusion that some form of mechanical proportioning and flow control device would be necessary in both experimental and possible commercial applications. In the

first place the concentration of the brine or liquor in the system is determined by the coil temperature. Secondly, the maximum concentration of the liquor leaving the heater is determined by the solubility of the salt or non-aqueous phase at the temperature prevailing in the freezer. The water available for evaporation therefore lies between the liquor concentration necessary for defrosting the coils, and the solubility of the salt or freezing point depressant at low temperature. Since, in practice, a wide variety of freezing point depressants and coil temperatures may be used, it is essential to provide means for adjusting the proportion of the liquor to be evaporated. It is also necessary to provide means for altering the rate of flow through the heater section, in accordance with the amount of water to be evaporated per unit time to maintain the desired relative humidity, without altering the proportion to be evaporated. Finally the rate of flow and the heat input must be balanced under all conditions to ensure maximum efficiency.

The regulation of the proportion of water to be evaporated and the rate of flow, while maintaining a balance between the heat input and the water to be evaporated, was accomplished by means of the equipment shown in Fig. 1. A small fraction of the liquor returning from the cooling coils to the supply tank through pipe A, is held in the trap B, which provides a small liquid head and permits reasonably close control of the flow entering the apparatus through valve C. This small flow enters the feed unit reservoir D through a screen and fills the reservoir to the level of the port E in the adjustable overflow standpipe F, which drains into the supply tank. Enclosed in the feed unit reservoir is the proportioning cylinder, G, which fills to the same level as that of the feed unit reservoir through the port H, the air escaping through the vent I. The piston J is provided with holes K, and carries an extension L, ground to cover the port M in the bottom of the cylinder. When the piston is raised about ½ in. by the activating mechanism to be described later, it acts as a slide valve to close port H, and opens port M. This allows the cylinder to drain into the heat exchanger N, and prevents the liquid in the reservoir from entering the cylinder during the drainage period. The quantity of liquid delivered by this cylinder can be altered by means of the adjusting screw O, which raises or lowers the liquid level in both the reservoir and cylinder.

The concentrated liquid returning from the heat exchanger N, enters the discharge unit reservoir DR, through the screened port P, attaining the same level as that in the heater Q. The amount of liquid contained in this cylinder R, is determined by the position of the displacement piston S, which can be adjusted, by the screw, T, so that the desired volume can be discharged from the cylinder. Otherwise this cylinder acts in the same manner as the feed unit cylinder, liquid entering through the port U, and air escaping through the vent V. Raising the piston ½ in. closes the port U, and opens the port W, allowing the concentrated liquor in the cylinder to discharge into the supply tank.

The entire mechanism is activated by means of a 1/75 h.p. motor X, with built-in reducing gear. This motor rotates the wheel Y, carrying the pawls Z, at a rate of 4 revolutions per hr. The pawls engage with the beam, BM, periodically, raising the auxiliary beam, AB, and the connecting rods carrying the pistons the desired distance. The pawls hold the pistons in the raised position for about 40 seconds to allow both cylinders to drain after which the pawl disengages. The pistons are then forced down by springs, and remain down for at least 80 seconds to allow the cylinders to refill, when they are again discharged by the engagement of the next pawl. As a rule the feed cylinder is adjusted to deliver about ¼ lb. of liquor, and the

intermittent delivery and removal of such small quantities of liquid has been found to have no appreciable effect on the performance of the unit.

In practice the amount of water that can safely be evaporated from the liquor is computed from the concentration necessary for defrosting and the solubility of the freezing point depressant used. If this should happen to be 30 per cent by weight, the feed and discharge cylinders are adjusted to deliver and remove 100 and 70 grams respectively. The rate of flow can then be varied, without altering the proportions by varying the number of pawls in the wheel Y, since these determine the number of activating impulses the pistons receive in unit time.

The only thing that remains is to establish a balance between the heat input and the amount of water to be evaporated. This is accomplished by means of a float, FL, in the discharge reservoir, DR. If the heat input is inadequate to evaporate the amount of water, as determined by the proportion and rate of flow, the liquid level in the system gradually rises. This lifts the float and opens the circuit to the motor X, and prevents the addition of more liquid until the level falls. Conversely, if the heat input is capable of evaporating more water than that supplied, the liquid level in the system falls and the float opens the heater circuit, until the normal level has been restored.

The remainder of the unit requires little explanation. The liquor in the supply tank is circulated over the cooling coils by means of the pump and returns to the supply tank by gravity. The liquid enters the insulated heater Q, containing the heating element, EL, through the heat exchanger, and the steam escapes through the opening, ST. In the experimental tests it has been found beneficial to bubble a small quantity of air through the jet, JT, to prevent the liquor from "bumping." If the steam is merely allowed to escape, condensation occurs to some extent on nearby objects. This is largely prevented by the passage of compressed air through the jet, VA, which induces a circulation of the room air through the ports PT, and nozzle NZ. This air cools the steam, dissolves part of it, and also serves to distribute the resultant "fog" throughout the room. If the air volume induced by the injector is carefully regulated the condensation that occurs in the nozzle is of little consequence. The small volume of air required for the jets, JT and VA, can conveniently be supplied by close coupling a small air pump to the otherwise free end of the pump motor.

The entire unit as shown in Fig. 1, requires about 4 sq. ft. of floor space, consumes about 1500 watts as a maximum, and is capable of evaporating about 1.5 lb. of water per hr., or sufficient moisture to raise about 42,000 cu. ft. of air from 65 to 100 per cent relative humidity at 10°F.

Moisture Delivered and Relative Humidity Attained

BEFORE discussing the practical aspects of numidification, it is of interest to deal with certain fundamental considerations. Although this equipment is capable of delivering relatively large quantities of moisture into the air in the storage space while preventing the formation of ice on the coils, it must be recognized that similar quantities of moisture are being removed from the air by the liquor or brine distributed over the coils. In other words, at a constant relative humidity the quantity of moisture condensed from the air per unit time is equivalent to the quantity added per unit time.

It might reasonably be expected that as the relative humidity of the air approached saturation, the condensing power of the brine on the coils would become extremely large and the evaporation and energy input necessary to

maintain these conditions correspondingly large. If this were true, the maintenance of the desired humidity of 95 per cent or higher might not be feasible economically.
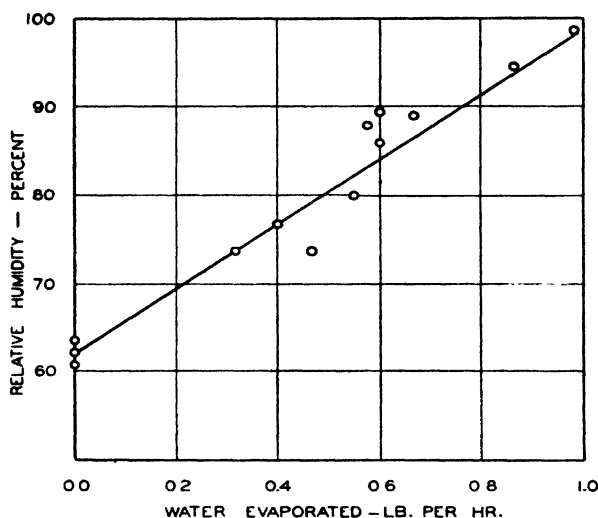
Reconsideration of this problem shows, however, that the resultant relative humidity of the room air depends on the rate at which moisture is delivered by the equipment and removed by condensation at the coils. Now the rate of condensation on the coils, in common with similar forms of sensible or latent heat transfer, is likely, as a first estimate, to be a linear function of the difference in poten- tial, in this case a condensation potential proportional to the difference in vapor pressure. For example, if the vapor pressure at the coil surface were equivalent to a relative humidity of 60 per cent in the room air, and in a given space the addition of 0.5 lb. of water vapor per hr. in- creased the humidity to 80 per cent, one might reasonably expect that the addition of one lb. of water vapor per hr. would raise the humidity to about 100 per cent.

With the equipment described above, it is possible to obtain an estimate of the amount of water evaporated per unit time, and at constant relative humidity, the moisture condensed out per unit time. Some experimental results are shown in Fig. 2, in which the observed relative hu- midity has been plotted against the water vapor delivered into the experimental room in pounds per hour. The re- sults are somewhat irregular partially due to the difficul- ties of maintaining an absolutely constant room tempera- ture, and a uniform rate of moisture delivery over the period required to make the dewpoint determinations. Al- though these experiments will have to be repeated to obtain more adequate quantitative information, the present results indicate that a linear relation exists between the amount of moisture delivered to the air and its relative humidity. These results show that although more energy must be added to attain high humidity levels, it is not a physical impossibility to obtain a practically saturated atmosphere in a freezer. In fact, it has been possible in individual experiments to fill the room with fog showing that the rate of humidification can exceed the rate of condensation on the coils.

Performance of Equipment

RETURNING to the practical aspects of the problem, it is evident that the entire heat input to the heater is added to the refrigerating load. In fact, any method of humidification where the energy necessary for evaporation is supplied from outside the freezer, such as the introduction of water or steam, increases the refrigerating load to that extent. This statement would not apply to humidification by the introduction of ice since the heat of sublimation of the ice comes from the freezer itself. However, it has already been pointed out that this procedure, although helpful, is incapable of maintaining the high humidities necessary. In fact, the introduction of water vapor in any form is incapable of maintaining relative humidities approaching saturation, unless the cooling coils are kept reasonably clear of ice. Intermittent defrosting, either by mechanical or other means, not only results in a lowered efficiency of the refrigerating machinery over the periods that the coils are coated with ice, but also results in an additional load of at least 144 Btu. per lb. of ice discarded on defrosting. The extent to which refrigerating costs are increased by reduced rates of heat transfer at the coils, resulting from ice formation, and by the wastage of ice on defrosting, varies from freezer to freezer and cannot be estimated accurately. The present method of humidification, involving continuous defrosting of the coils, avoids these losses, and although the magnitude of the resulting saving is unknown, it is probably more than adequate to offset the energy necessary for operating the small pump motor required for circulating the brine.

Before discussing the results of a series of experiments conducted recently, it should be mentioned that certain preliminary tests indicated that the heat input necessary to attain 95 per cent relative humidity decreased with the temperature difference between the cooling coils and the air, and also decreased as the temperature of the freezer decreased. These results are in accordance with theory, since the condensing power of the coils should decrease as the condensing potential between the coils and the air

Table 1. Efficiency of Humidifying Equipment Under Various Operating Conditions in a Room of 1125 cu. ft. with a Coil Temperature of −5° F.

Room temperature, °F.	Relative humidity, %	Rate of liquid delivery to apparatus, lb./hr.	Proportion evaporated by wt., %	Heat input, Btu./hr.	Heat of vaporization, Btu./hr.	Vaporization efficiency, %	Overall efficiency corrected for condensation, %
12.0	74	3.94	9.0	745	380	51	49
14.3	80	3.93	15.9	1785	671	(38) ?	(33) ?
14.5	87	3.94	16.7	1434	706	49	43
13.5	88	3.71	17.7	1448	707	49	43
13.6	90	3.75	17.5	1505	703	47	42
15.0	90	3.86	18.9	1789	785	44	39
14.0	95	3.85	24.7	2247	1020	46	41

is reduced. Again, as the air temperature is lowered, its moisture carrying capacity is reduced, and a smaller amount of water vapor, and consequently less heat, is required to raise the humidity to 95 per cent. As yet, we cannot say with assurance that there is a direct proportionality between the water to be evaporated and the air temperature and the temperature gradient between the coils and the air, but since it is theoretically sound and

supported by the results of preliminary tests, it will be assumed to be true until further information is available.

In view of the difficulties involved in making precise measurements under the conditions existing in a freezer, the first series of systematic experiments was made under conditions of relatively high freezer temperature with relatively large temperature gradients between the coil and air. These conditions exaggerated the difference between the conditions under test, but are naturally those requiring the maximum heat input to attain the desired relative humidity.

The results of these experiments, made with a calcium chloride brine having a density of 1.200, appear in Table 1. The mean temperature of the cooling surfaces was kept at —5.0°F. throughout, while the temperature in the freezer, although relatively constant during a given experiment, varied from 12 to 15°F. between different experiments. The temperature gradient between the coils and the air therefore varied from 17 to 20°F., which is about twice the differential necessary in practice for maintaining the desired temperature. The relative humidity varied between 74 and 95 per cent, as determined by the heat input. The quantity of liquor supplied to the heater was about 3.9 lb. per hr., and the proportion of this evaporated was varied from about 9.0 to about 25 per cent by weight. The total heat input to the heater, and the heat required to evaporate the water at room temperature is the only portion of the energy that does useful work, while the remainder represents the sensible heat in the steam above room temperature, sensible heat losses through the heat exchanger, and other sensible heat losses from the equipment as a whole.

The efficiency of vaporization was therefore computed by expressing the heat necessary for vaporization as a percentage of the total heat input. Table 1 shows, with the exception of one doubtful value, that the vaporization efficiency usually lies between 45 and 50 per cent. The sensible heat contained in the steam above room temperature accounts for about 10 per cent of the remaining heat input. This source of loss cannot be reduced, since any arrangement of heat exchange with the steam causes condensation. The remaining loss of 40 per cent of the heat input might reasonably be reduced, by providing a more efficient heat exchanger, better heat insulation, or actually locating the heater and heat exchanger outside the room. It is felt that the vaporization efficiency might easily be raised from 50 to 65 per cent, a value actually attained in individual experiments, and this would effect a significant reduction in the heat input necessary for humidification.

In practice a certain amount of the steam delivered by the heater condenses in the delivery nozzle, etc., and the quantity of water vapor delivered to the air is thereby reduced. By measuring this source of condensate, it was possible to obtain an estimate of the overall or humidification efficiency. In general, this is about 5 per cent lower than the vaporization efficiency and represents a source that might reasonably be reduced.

The results in Table 1 as a whole show: that it is possible to attain 95 per cent humidity in a freezer at 14°F. with coils at —5°F.; and that the efficiency of the apparatus is not affected beyond experimental error, by the possible variations in the proportion of water to be evaporated from a calcium chloride brine of density 1.20. The effect of other factors, such as the rate of flow, type of heat exchanger, etc., have yet to be investigated, but it appears that the overall efficiency can reasonably be increased to about 60 to 65 per cent.

THE practical operator is naturally interested in the
extent to which humidification to 95 per cent relative
humidity will increase his present refrigeration load.
So far all estimates of the heat to be eliminated under
various conditions have been made in the experimental
room of 1125 cu. ft. and the relative proportions of heat to
be eliminated to maintain the desired temperature, and
to compensate for humidification, may reasonably be some-
what different in a large freezer room. Again our experi-
ments are as yet incomplete and the tests which we have
conducted have been made under conditions demanding a
relatively large heat input for humidification.

Nevertheless, it is of interest to examine the economic
practicability of humidifying a freezer to 95 per cent rela-
tive humidity. Reference to Table 1 shows it was necessary
to add 2250 Btu. per hr., at 41 per cent overall efficiency,
to humidify the space to 95 per cent relative humidity.
The heater on the equipment was then shut off, and with
all auxiliary motors and lights operating, and the same
number of operators in the room, it was found that the
maintenance of 15°F. required the elimination of 4690
Btu. per hr. Under these conditions the additional heat
necessary for humidification represents about a 48 per
cent increase in the refrigerating load. By reducing the
temperature gradient between the coils and the air from
about 20°F. as used in these experiments, to 10°F. as
commonly used in practice, the condensation potential at
the coil surface could be reduced to about half, with a
similar reduction in the heat input necessary for humidifi-
cation. Under these conditions the heat input for humidi-
fication would be about 24 per cent.

Again, it seems likely that freezer temperatures in the
region of 0°F. will be necessary to prevent detrimental
chemical changes in certain products. Since the moisture
carrying capacity of air at 0°F. is about half that at
15°F., the amount of heat necessary for evaporation
should be reduced proportionately. If this assumption is
correct, the heat input necessary for humidification to
95 per cent at 0°F. with coils at −10°F. should only be
about 12 per cent greater than that required to maintain
the temperature condition. Again, if the efficiency of the
equipment can be increased from 41 to about 60 per cent,
the increase in the refrigerating load would be less than
10 per cent.

These deductions, based on assumptions from theoretical
considerations, indicate that humidification of commercial
freezers should be economically practicable. Experiments
are now under way to test these assumptions, and although
the results of individual tests indicate that they are gen-
erally correct, further work is necessary before a definite
statement can be made. Nevertheless, it appears that a
reduction in the drying power of the air can be effected
more economically by humidification than by a reduction
in temperature. Taking a relative humidity of 80 per
cent as the prevailing humidity in freezers, it would be
necessary to maintain temperatures below −20°F. in order
to reduce the drying power to the extent prevailing in
spaces held at 0°F. and 95 per cent relative humidity.
Such a decrease in temperature would represent about a
30 per cent increase in the refrigerating load even if the
reduced capacity of the compressor at low temperature is
neglected.

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FROZEN STORAGE OF POULTRY. II. BLOOM [1,2]

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The term "bloom" is used to designate the fresh surface appearance of meats. Lack of bloom does not necessarily imply spoilage or loss of flavor, but the consumer generally prefers products with good bloom. Successful storage practice must therefore aim to preserve bloom as well as to prevent deterioration through spoilage.

With poultry both the over-all quality and the bloom shortly after slaughter may vary considerably between different birds and it seems probable that poultry from the various commercial grades may suffer a differential loss of bloom during storage. This study was undertaken not only to determine the effect of various storage conditions on the retention of bloom, but also to determine whether poultry of different initial quality suffered a differential loss of bloom during storage in the frozen state.

Since bloom may be affected by many factors and cannot be defined objectively, it is generally assessed subjectively from the color and opacity of the superficial layers. Since Griffiths, Vickery, and Holmes (1932) have reviewed this subject in connection with an extensive study of bloom in lamb carcasses, a detailed discussion is unnecessary here. Briefly, these investigators report that darkening of muscle by methemoglobin formation is unlikely to occur to any appreciable extent at storage temperatures below —10°C.(14°F.). Apparently fats are more likely to change color, since Vickery (1932) reports that yellowing of rabbit fat may occur at —10°C. and recommends temperatures of —14°C.(6.8°F.) or lower to prevent this change. Since poultry is ordinarily stored at temperatures below —12°C.(10.4°F.), on this continent, it seems unlikely that any loss of bloom would result from a direct change in either the muscle or fat pigments.

Griffiths, *et al.* found that the main cause of loss of bloom was an increase in the opacity of the superficial tissues, which indirectly affects the color of the visible tissue. During freezing an increased opacity was obtained when the *rate* of evaporation was high, but

during storage the loss of bloom was approximately proportional to the *extent* of evaporation.

The factors affecting the rate and extent of evaporation in relation to the surface desiccation or freezer-burn in frozen poultry have been discussed in an earlier paper in this series by Cook (1939b). If loss of bloom in poultry is determined by evaporation, as it is in frozen lamb, it is to be expected that loss of bloom and freezer-burn will be associated. Nevertheless, these two conditions must not be confused. The increased opacity, causing loss of bloom, results from a uniform evaporation from the entire skin surface, whereas freezer-burn results from the severe desiccation of irregular areas. Naturally the latter condition has a serious effect on the appearance and may therefore be said to affect the bloom. In these studies, however, the bloom was assessed only from the appearance of areas unaffected by freezer-burn.

EXPERIMENTAL PROCEDURE

The poultry used in these studies consisted of birds killed and packed in the laboratory and birds killed in commercial packing plants. Dressing methods employed included dry plucking, semi-scalding, and wax dressing, but no effort was made to determine the relative effects of these various methods on bloom. The birds were precooled for 24 hours, graded, and packed at a temperature near the freezing point. Most of the poultry was placed in the freezing rooms within 48 hours after slaughter. All of the product was frozen at the same temperature as that at which it was subsequently stored.

Since no suitable objective method has yet been devised for estimating bloom, the method of subjective scoring, described in a previous paper by Cook (1939a), was used. Five grades of bloom were defined and each allotted a whole number. As the breast was scored separately from the legs and other visible parts of the body, the summation of these independent scores resulted in a possible score of 120 points per box of 12 birds. The figures subsequently reported for box lots of poultry are expressed on this basis and represent the mean scores of two judges.

In the previous study, Cook (1939a), it was observed that the most serious source of error in scoring arose from time-to-time variations in the inspectors' judgments. An attempt was made in the present investigation to estimate this error by scoring certain boxes on two successive weeks at the beginning and end of the storage period, on the reasonable assumption that during short intervals little change in bloom was to be expected. The differences between these successive scores enabled the magnitude of the time-to-time variability of the judges to be estimated by statistical methods.

Unfortunately, duplicate inspections throughout the entire storage period could not be tolerated, since frequent opening of the boxes would have affected the humidity in the package, hence the rate of evaporation.

EFFECT OF HUMIDITY AND TEMPERATURE ON BLOOM

Two birds, not initially scored for bloom, were stored at each of a number of relative humidities at two storage temperatures, as described by Cook (1939b). After 83 weeks' storage, the breasts of the birds were scored, yielding the values reported (Table 1) which

TABLE 1

Effect of Humidity and Temperature on Bloom

Stored at —13 5°C (7.5°F.) for 83 weeks			Stored at —22°C (—7 5°F.) for 83 weeks		
Computed mean effective relative humidity	Mean bloom score (possible=5)	Proportion of skin area freezer-burned	Computed mean effective relative humidity	Mean bloom score (possible=5)	Proportion of skin area freezer-burned
pct.		pct	pct.		pct.
90	2.00		89	3.25	
92	3.25	10 to 25	91	3.75	5 to 10
94	3.00		93	4.25	
96	3.00	5 to 10	96	3.75	....
98	4.50	Slight	98	4.00	None
100	5.00	None	100	4.50	None
General mean at each storage temperature.......	3.48	......	......	3.92	Difference =.44

Necessary differences for significance		
	5% level	1% level
Between mean of duplicates...	.50	.65
Between general means at each storage temperature................	.20	.28

are the mean scores for the two birds stored at each condition. It is evident that the bloom on the portion of the skin surface unaffected by freezer-burn decreased as the proportion of the freezer-burned area increased. In spite of the observed irregularities, some of which may be attributed to variations in the initial bloom of different birds, it is evident that loss of bloom is greatest under storage conditions that promote drying. Bloom is therefore retained best at high relative humidities and low storage temperatures, since these conditions reduce evaporation.

This conclusion was supported by the results of a poultry packaging experiment described in the first paper of this series—"Surface Drying of Frozen Poultry During Storage"—which was published in Vol. 4, pages 407 to 418 of *Food Research*. The birds were not scored

for bloom but it was observed that in packages which maintained relative humidities in the vicinity of 98 to 100 per cent, the product retained excellent bloom over a one-year storage period at —13.5°C. (7.5°F.). In packages having 85 per cent relative humidity or less, the skin area unaffected by freezer-burn was practically devoid of bloom. The bloom on the product contained in packages that maintained humidities between these extremes was rated "fair" or "good" when examined by practical poultry graders at the end of the storage period.

In order to determine the effect of storage temperature on bloom of poultry, one box of each of four commercial grades, to be described later, were stored at —13.5°C. and —22°C.(—7.5°F.) for a 27-week period. These birds were packed in 12-bird boxes in the usual manner, using unsealed liners of heavy waxed paper. All of the boxes stored at the higher temperature decreased in bloom more than those held at the lower temperature. The average decrease in the bloom score for all four boxes was 34 points at —13.5°C., and only eight points at —22°C. Freezer-burn was also more severe at the higher temperature. It appears therefore that the differential loss of bloom can be explained by the differential rates of evaporation at the two temperatures.

BLOOM IN RELATION TO QUALITY

To determine the relation between quality and change of bloom during storage, poultry of five Canadian commercial grades was used. These grades, in order of decreasing quality, are as follows: Milkfed A, Milkfed B, Selected A, Selected B, and Selected C. Birds having a white fat and skin are classed as "Milkfed," the letters A and B indicating the amount of fat, fleshing, and finish. Selected A birds are fat and well fleshed but, as their skin and fat have a yellow color, they are excluded from the "Milkfed" classes. The Selected B grade is well fleshed but lacks the fat and finish of the higher grades. Selected C, a still lower grade, includes birds that are excluded from the higher grades for many reasons.

All of the birds were graded by skilled inspectors before being packed in 12-bird boxes with unsealed, heavy waxed-paper liners. All subsequent scoring was for bloom only, the judges attempting as far as possible to exclude consideration of the other factors contributing to their quality as a whole. Similar periodic scorings were made at approximate monthly intervals during a storage period of about one year.

The results of these detailed examinations, covering some 20 boxes of poultry and storage periods that overlapped and extended over a two-year period, are summarized (Table 2). A few boxes

only of the Selected C grade were stored, and since the bloom scores allotted to different birds varied greatly, little confidence could be placed in the results obtained.

Before considering the results, it is necessary to obtain some estimate of the error and variability of the reported mean values. The variability in the inspectors' judgments between examinations was estimated from the scores alloted to a number of boxes on successive weeks at the beginning and end of the storage period. This quantity expressed as a standard deviation was found to be ± 5.86. To this must be added the variability in the bloom score between different boxes within the same grade. On the basis of the initial in-

TABLE 2

Loss of Bloom in Relation to Grade

Storage period	Bloom scores for various commercial grades (Canadian)			
	Milkfed A	Milkfed B	Select A	Select B
wk.				
0-10	116	111	111	104
10-20	112	106	106	101
20-30	96	101	91 [1]	96
30-40	98	86 [1]	85	87 [1]
47-56	92 [1]	84	72	69
Decrease in bloom score over storage period	24 [2]	27 [2]	39 [3]	35 [3]

[1] Significantly lower than initial score   [2] Five per cent level of significance   [3] One per cent level of significance.

spections this standard deviation was found to be ± 6.50. The values reported are therefore subject to a standard deviation of ± 8.76 arising from these two sources. The significance of the reported differences was tested, using this value and the number of degrees of freedom applicable to the comparison in question.

The initial scores awarded to the Selected C birds differed significantly from all the other grades, except the Selected B, but the differences between the initial scores allotted to the four highest grades were not statistically significant. The time required for the bloom to fall to a value significantly lower than the initial rating is indicated (Table 2). There is some suggestion that the bloom is lost more rapidly as the grade or initial quality decreases, but this cannot be stated with assurance, since the results with the Selected B grade indicate the reverse.

The decrease in bloom over the entire storage period is shown for each grade at the bottom of the table. These results are more conclusive since all are statistically significant. However, the decrease

on the yellow-colored "Selected" grades is much greater than on the white and better-finished "Milkfed" grades. Since it seems unlikely that a chemical change in the pigments could occur at a storage temperature of —22°C., the decrease in bloom must be attributed to an increase in opacity of the skin or subcutaneous layers as a result of evaporation. It appears, therefore, that a given amount of evaporation must alter the apparent color, or surface appearance, much more in poultry that is yellow or dark than in birds that are initially lighter colored.

SUMMARY

When dressed poultry is stored in the frozen state, the loss of bloom during storage depends mainly on the extent of evaporation. Low temperatures and high humidities tend, therefore, to preserve bloom. In a package lined with a sealed, water-resistant material, such as waxed paper, little deterioration of the bloom can be detected during 50 weeks' storage at —13.5°C.(7.5°F.), but if the liner is not sealed, serious deterioration occurs in from 20 to 30 weeks at this temperature.

Poultry of different grades stored in the standard wooden boxes with unsealed liners at —22°C.(7.5°F.) all showed a significant decrease in bloom over a storage period of about a year. There was some evidence that the initial bloom was retained longer on the higher grade birds. The over-all decrease in bloom during the entire storage period showed that the lighter-colored "Milkfed" grades suffered less in appearance than the more yellow "Selected" grades. It appears, therefore, that under the same storage conditions the initial color is as important as the over-all quality of the bird in determining the loss of bloom that will occur.

ACKNOWLEDGMENT

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FROZEN STORAGE OF POULTRY. III. PEROXIDE OXYGEN AND FREE FATTY ACID FORMATION [1]

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In earlier papers in this series by Cook (1939a, 1939b) it has been shown that physical changes, principally surface desiccation, may cause depreciation of frozen poultry stored over a period of a few months. These changes, however, primarily affect the appearance rather than the flavor or eating quality. Complaints of "storage" flavor are sometimes heard in connection with poultry that has been held in the frozen state. It seems probable that this may originate from chemical changes in the fats owing to oxidation and hydrolysis. Analyses were therefore made to determine the free fatty-acid and peroxide-oxygen values of fat from frozen poultry that had been stored under various conditions.

Lea (1934) has studied the oxidation and hydrolysis of the skin fat of poultry stored in air and carbon dioxide at chill temperatures, i.e., 0 to —1°C. (32 to 30.2°F.). His results suggest that poultry fat is comparatively stable toward oxidation and that the hydrolysis of the fat is negligible if the growth of microörganisms is reduced.

ANALYTICAL PROCEDURE

The free fatty-acid content was determined by a slight modification of the method mentioned by Lea (1933a). Two grams of fat, dried and extracted by a procedure to be subsequently described, was refluxed with 25 ml. of neutral ethyl alcohol for four minutes and titrated with .05N potassium hydroxide, using phenolphthalein as the indicator.

The determination of peroxide oxygen is based on the oxidation of potassium iodide by the active oxygen in the fat to liberate iodine. The results are expressed as the number of ml. of .002N sodium thiosulphate required per gram of fat to react with the liberated iodine. A preliminary investigation was made on chicken fat to compare Lea's (1929, 1931) procedure with the modification suggested by French, Olcott, and Mattill (1935). It was found that both gave essentially the same results. Since the latter procedure

[1] Issued as paper No. 32 of the Canadian Committee on Storage and Transport of Food.

requires less manipulation, however, it was used in all subsequent determinations.

Lea (1931) has pointed out that there are two inherent errors in this method. Although the amount of iodine liberated is determined by the peroxide oxygen present, that available for the titration depends first, on the quantity of fat present and second, on its degree of saturation. With a given fat the amount of iodine removed by absorption will be roughly proportional to the amount of fat in the solution. Likewise the amount of iodine available for the titration will be decreased more by a highly unsaturated fat than by a more saturated one.

Usually the peroxide-oxygen values reported in the literature are not corrected for these two errors and are consequently lower than the actual values. Since poultry fat is rather unsaturated (Lea 1934), it seemed desirable to determine the approximate magnitude of the error involved. This was done in a preliminary experiment by titrating solutions of various concentrations of the fat, plotting these values against concentration, and extrapolating to zero concentration. The results obtained with five fats, varying in peroxide-oxygen content from 4.8 to 43.4 ml. .002N sodium thiosulphate per gram of fat, showed that the actual values were about 20 per cent higher than the observed. This correction was not applied to the results of subsequent experiments, since these could only be interpreted on a relative basis and consequently would be unaffected by any constant percentage error.

EFFECT OF METHOD OF DRYING AND EXTRACTION

Since the peroxide-oxygen content of a fat may increase during its extraction from the product, some attention was given to methods that would minimize this change. The samples of fat obtained from the skin and subcutaneous fat layers of the frozen birds were chopped up as finely as possible in the freezing chamber in which the product had been stored. Little or no change in the peroxide-oxygen level could therefore have occurred at this stage.

The three following procedures were tested for drying this material and extracting the fat:

Procedure 1. Dried *in vacuo* at 50°C.(122°F.) followed by—A. Soxhlet extraction in a darkened room at 4.5°C.(40°F.), B. Soxhlet extraction in laboratory (diffuse daylight) at 25°C.(77°F.) (approx.).

Procedure 2. Mixed with anhydrous sodium sulphate, allowed to stand in the dark, and extracted as under A and B above.

Procedure 3. Mixed with anhydrous sodium sulphate, and petroleum ether added immediately so that the drying and extraction proceeded simultaneously during standing in a darkened room at 4.5°C.

Three such experiments were made, using three different poultry fats and drying periods varying from one to three days. The petroleum ether used for extraction was redistilled and tested for the presence of oxidizing substances. After extraction for approximately three hours, the petroleum ether was removed by heating on a steam bath followed by heating *in vacuo* at 50°C. (122°F.).

The results of these experiments (Table 1) indicate that in order to decide on the best procedure it is necessary to assume that the method giving the lowest values caused the least alteration in the fat. Although the initial peroxide-oxygen content of the fatty material used in the first experiment was zero with all drying and extraction procedures, it is evident from the results that the extracted fat prepared by Procedure 2 was less stable than that prepared by the other two procedures. The fatty material used in the second experiment contained peroxide oxygen immediately after extraction. In this instance the sample prepared by Procedure 2A had the minimum value, while that prepared by Procedure 2B yielded the highest peroxide-oxygen values observed. Although there is little to choose between Procedures 1 and 3, the indications are that Procedure 1 is superior. The fat used in the third experiment had a slight but definite peroxide-oxygen content. All three procedures gave essentially the same results.

On the whole the results indicate that the method of drying used in Procedure 2 is inferior to the other two tested. Although the results (Table 1) show some irregularities, in general, extraction in a darkened room at 4.5°C. yielded lower peroxide-oxygen values than that under ordinary laboratory conditions. In consequence the fat used in all subsequent determinations was obtained by drying *in vacuo* at 50°C. followed by extraction in a darkened room at 4.5°C.

EFFECT OF STORAGE CONDITIONS

The first series of experiments was comprised of analyses on fat obtained from poultry stored in commercial packages for various periods under different temperature conditions. The fatty material consisted mostly of the skin wherever it appeared to contain an appreciable quantity of fat. In the better grades, one bird usually yielded sufficient fat to make the necessary determinations. In the grades of lower quality, however, it was necessary to combine the fatty material from two birds to obtain sufficient fat. Duplicate

TABLE 1

Effect of Method of Drying and Extraction on Peroxide Oxygen[1] and Stability of Fat

Procedure	Method of drying	Extraction — Method	Extraction — Conditions	Experiment 1. Fat A. Drying period 2 days — Exposure period—days			Experiment 2. Fat B. Drying period 1 day — Exposure period—days		Experiment 3. Fat C. Drying period 3 days — Exposure period—days
				0	7	14	0	1	0
1	In vacuum oven at 50°C.(122°F.)	Soxhlet	In darkened room at 4.5°C.(40°F.)......	0	0	1.0	6.5	31.0	1.5
	In vacuum oven at 50°C.(122°F.)	Soxhlet	In laboratory at 25°C.(77°F.)......	0	0	1.2	7.3	28.0	2.2
2	Mixed with anhydrous sodium sulphate.	Soxhlet	In darkened room at 4.5°C.............	0	15	113	4.4	19.0	2.4
	Mixed with anhydrous sodium sulphate.	Soxhlet	In laboratory at 25°C.............	0	27	121	9.1	42.0	1.3
3	Mixed with anhydrous sodium sulphate, petroleum ether added immediately.	Decanted at end of drying period	In darkened room at 4.5°C.............	0	0.2	1.6	8.1	11.0	2.0

[1] Given as ml. .002 N. $Na_2S_2O_3$ per gram of fat.

extractions were made on the fat from different birds under each storage condition, and duplicate analytical determinations were made on each extract (Table 2).

Several of the boxes were stored for one period at one temperature and then held for another in a room at —40°C.(—40°F.) before the analyses were made. Since the changes in the fats should be practically negligible at this low temperature, this phase of the stor-

TABLE 2

Effect of Storage Conditions on Peroxide Oxygen and Free Fatty Acid in Poultry Fat Packed in Commercial Boxes

Storage		Peroxide oxygen	Free fatty acid as oleic acid	Remarks
Temperature	Time			
°C	mo.	ml. .002N $Na_2S_2O_3$ per gm. fat	pct.	
—13.5	6	0	0.42	5 to 20% of skin area freezer-burned.
—13.5	6	0	0.52	5 to 20% of skin area freezer-burned.
—13.5	12¾	2.77	0.94	0 to 5% of skin area freezer-burned.
—13.5	12¾	2.53	1.6	Rel. hum. in package = 98%.
—13.5	12¾	2.47	0.63	50 to 80% of skin area freezer-burned.
—13.5	12¾	2.06	0.70	Rel. hum. in package = 80%.
—13.5	21	1.01	0.38	20 to 25% of skin area freezer-burned.
—13.5	21 [1]	0.82	0.57	20 to 25% of skin area freezer-burned.
—22.0	6	0	0.93	No freezer-burn.
—22.0	7 [2]	0.45	0.96	No freezer-burn.
—22.0	10½ [3]	0.82	0.62	. .. .
—22.0	10½ [3]	0.89	0.51	....
—22.0	15½	0.48	0.67	10 to 50% of skin area freezer-burned.
—22.0	15½	0.84	0.58	10 to 50% of skin area freezer-burned.

[1] Subsequently stored at —40°C (—40°F) for 6 months before analysis was made [2] Subsequently stored at —40°C. for one year before analysis was made [3] Previously stored for 6 weeks at —13 5°C.(7.5°F.) and subsequently for 6 months at —40°C

age history is merely indicated under "remarks." The relative humidity of the atmosphere surrounding the product in the package was measured in only a few instances; however, the area of skin affected by freezer-burn is reported for most of the samples.

The results show that little or no peroxide oxygen was formed within six months at either —13.5 or —22°C.(7.5 or —7.6°F.). Longer periods of storage gave average values of about 2 and .75 ml. .002N sodium thiosulphate at —13.5 and —22°C., respectively. Although the results indicate that more peroxide-oxygen formation occurred at the higher than at the lower temperature, this cannot be stated conclusively, since the fat from birds stored for an extended

period of one temperature in some cases had less peroxide oxygen than others stored under the same conditions for shorter periods. It appears therefore that the fat of different birds varies in its susceptibility to peroxide-oxygen formation. Similarly the amount of free fatty acid varied from .38 to 1.6 per cent as oleic acid between different birds, but without evident relation to the storage conditions.

TABLE 3

Effect of Temperature and Humidity on Peroxide Oxygen and Free Fatty Acid in Poultry Fat

Storage			Peroxide oxygen	Free fatty acid as oleic acid
Temperature	Relative humidity	Time		
°C.	pct.	mo.	ml. .002N Na₂S₂O₅ per gm. fat	pct.
—13.5	<82 to 90	25	5.08	.75
—13.5	<82 to 90	25	8.16	.64
—13.5	92 to 94	25	8.18	.56
—13.5	92 to 94	25	7.19	.58
—13.5	96 to 98	25	4.46	.51
—13.5	96 to 98	25	6.48	.53
—13.5	100	25	2.18	.57
—13.5	100	25	1.54	.88
—22.0	<76 to 88	25	0	.60
—22.0	<76 to 88	25	1.90	.55
—22.0	91 to 93	25	1.36	.54
—22.0	91 to 93	25	0.85	.58
—22.0	96 to 98	25	1.00	.49
—22.0	96 to 98	25	0.87	.41
—22.0	100	25	0	.45

In the second experiment two birds were stored at seven relative humidities at both —13.5 and —22°C. for 25 months in order to obtain more definite results. The method employed for controlling the relative humidity has been described in an earlier paper by Cook (1939a). When the analyses were undertaken it was found that the fat from one bird was inadequate for the determinations. Had the two birds stored under each condition been used to obtain the necessary material, it would have been difficult to separate the effect of different storage conditions from the variation between birds. In consequence the birds stored at two successive relative humidities, starting with the lowest, were treated as a group, i.e., the fat from one bird stored at a certain relative humidity was mixed with the fat from a bird at the next higher humidity, in order to provide sufficient material for the analysis. In this way two similarly treated

birds were left for a duplicate extraction. This procedure gave less information on the effect of humidity but enabled the variable behavior of the fat of different birds to be evaluated.

The results of these experiments (Table 3), although somewhat irregular, indicate that peroxide-oxygen formation decreases as the storage temperature is lowered and as the relative humidity increases at constant temperature. In order to determine the significance of the observed differences the results were subjected to an analysis of variance. The variance attributable to experimental error was computed from the difference between duplicate titrations on the same extract, and that attributable to the difference between birds was computed from the two extractions made on birds stored under the same conditions. In the complete results of this analysis (Table 4)

TABLE 4

Analysis of Variance of Peroxide Oxygen Content of Poultry Fat

Variance attributable to	Degrees freedom	Mean square
Experimental error	15	0.0216
Difference between birds	7	2.694 [1]
Difference between storage humidities	6	13.133 [2]
Difference between storage temperatures	1	154.724 [2]

[1] Indicates one-per cent level of significance   [2] Indicates five-per cent significance

it is evident that all of the possible differences are significant with respect to differences between birds. The effect of temperature exceeds that of humidity, which in turn exceeds the difference between different birds.

The results of the free fatty-acid determinations reported (Table 3) show that the fat from all of the birds was remarkably similar with respect to this constituent. Some slight variations are evident, but, since they were not related to the storage conditions, no statistical analysis was made.

CONCLUSIONS AND DISCUSSION

The results show that the free fatty-acid content of poultry fat after storage varies somewhat between birds, but is usually low, and shows no relation to the storage conditions at freezing temperatures.

The storage temperature is the most important factor determining the extent of peroxide-oxygen formation in poultry fat, the amount increasing with increase in the storage temperature. At constant temperature, low relative humidities accelerate peroxide-oxygen formation. This is presumably the result of surface drying or freezer-burn which exposes more of the fat to the air. These two

are the most important factors determining the condition of the fat of poultry following storage in the frozen state. However, it has also been shown that the fat from different birds varies in its susceptibility to oxidation.

Even after prolonged storage (25 months) at —13.5°C.(7.5°F.) the maximum peroxide-oxygen titration observed was only about 8 ml. of .002N sodium thiosulphate per gram of fat. Correction for iodine removal by the fat would increase this some 20 per cent, but such corrections are without significance since the point at which a rancid odor or flavor becomes evident in poultry fat is unknown. Lea (1933b) reports that bacon fat becomes rancid when the peroxide-oxygen titration reaches 8 to 10 ml., and suggests (1934) that even higher values may be required before rancidity can be detected in poultry fat. This, together with the fact that the extracted fats all appeared to be sweet, indicates that probably the fat of none of these birds would have been regarded as rancid. However, the incipient changes indicated by the higher peroxide-oxygen values might have caused a loss or change of flavor.

It is somewhat difficult to assess the commercial significance of these results. Since the fat from poultry stored for a year or more at temperatures of —13.5°C.(7.5°F.) usually showed peroxide-oxygen values of less than three ml., it is concluded that this fat is normally quite resistant to oxidation and that storage temperatures of —13.5 to —18°C.(7.5 to 0°F.) are quite adequate for preventing this change over the normal storage period. At these temperatures, however, high relative humidities are necessary to prevent direct depreciation from freezer-burn, as shown by Cook (1939).

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STUDIES ON THE BACTERIOLOGY OF WILTSHIRE BACON[1]
I. METHODS FOR QUANTITATIVE ANALYSIS OF CURING PICKLE

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Since 1934, studies on bacon-curing problems have been carried on by the Division of Bacteriology, Science Service, in coöperation with a local packing plant. Chief interest has been directed toward a fundamental study of the microörganisms associated with the curing of Wiltshire sides. The following report deals with the development of methods for analysis for Wiltshire pickle.

Studies on the relation of bacteria to salt have produced a voluminous literature which will not be summarized here. Much of our knowledge of the effect of salt on bacteria has been derived from investigations of bacterial life in brines, extensively reviewed by Hof (1935), and from studies of the deterioration and discoloration of fish, hides, or other products cured in salt or strong brines. The media employed have been very diverse in character and devised chiefly with the object of isolating and cultivating halophilic organisms. Thus, Hof (1935) isolated organisms from salt environments by means of saline enrichment cultures on standard laboratory media and on a bean-extract medium containing from 12 to 18 per cent sodium chloride. LeFevre and Round (1919) isolated organisms concerned in the fermentation of cucumber pickles by means of a cucumber-juice medium containing 10 per cent added salt. Schoop (1929) developed a modified fish bouillon containing 15 per cent sodium chloride for the examination of canned salt fish. Clayton and Gibbs (1927) prepared peptone, fish, and rice-water medium with 20 per cent added salt for the isolation of halophiles. Lochhead (1934) developed a milk-salt (20-25 per cent) agar medium for the isolation of halophiles from discolored hides. Numerous references to the bacteriology of salt lakes and sea water reveal many modifications of saline media. Most of the experimental work has been directed towards the isolation of specific bacterial types rather than to a study of the total flora and quantitative relationships at different salt strengths.

Little has been published on the bacteriology of meat-curing pickles, although several reports of ham souring and related problems occur in the literature. Tanner and Evans (1933a, 1933b, 1934a, 1934b), studying the effect of meat-curing solutions on anaerobic bacteria, have shown that sodium chloride possessed the greatest activity as a preservative and that neither sodium nitrite nor sodium nitrate alone in the concentrations used commercially had much effect. Four commercial curing mixtures failed to inhibit growth or toxin formation when they were used in the proportions called for in the formulae. Sturges (1923) reported that ham-curing solutions contained a heterogeneous flora even though 15 per cent salt was present. Sturges and Heideman (1924) later classified the bacteria found in curing pickle according to their salt tolerances. Horowitz-Wlassowa (1931) studied the organisms in meat-curing brine and reported a count of 10,000 per ml. in old pickle. In most cases nutrient agar medium was used, with or without the addition of salt or curing pickle. Little information is available as to the effect of salt concentration or other factors concerned with the quantitative estimation of organisms from pickling brines. Accordingly, the present paper summarizes experience gained during five years' study of the estimation of bacteria in curing pickle.

EXPERIMENTAL PROCEDURE

Preparation of Media: Difco nutrient agar was used as the basic medium from which 5-, 10-, and 15-per cent salt agars were prepared on a weight-volume basis. All media were standardized to pH 6.7 and filtered prior to sterilization. For counts by the dilution method, Difco nutrient broth and a broth containing .1 per cent yeast-extract and .5 per cent tryptone were tested. Both contained 10 per cent sodium chloride in addition to .1 per cent potassium nitrate or .002 per cent potassium nitrite. To obtain semi-solid media from the above, .1 per cent agar was added. In addition, preliminary tests were made with pork and pickle infusion agars. The former was prepared from 500 grams of fresh pork or from a commercial desiccated product, and the latter by adding 50 per cent by volume of sterile pickle (Seitz filtered) to an equal volume of hot, sterile three-per cent agar.

Effect of Incubation Period on Development of Colonies: In preliminary tests at incubation temperatures of 37, 20, and 5°C.(98.6, 68, and 41°F.) it was found that maximum counts were obtained on all media at 20°C. The rate of colony development on media containing increasing quantities of salt varied with the salt concentration

(Table 1), where maximum colony development is taken to be 100 for each medium.

In preliminary experiments with 20- and 25-per cent salt agars the lag in colony development was so great that under normal conditions of incubation salt crystals formed and made counting very difficult. This, and the fact that bacteria counts were in all cases lower on the 20- or 25-per cent salt agar than on 10- or 15-per cent salt, precluded further analyses with these media. Although maximum counts were not obtained on nutrient agar plates until the third day at 37°C. or the sixth day at 20°C., it was decided to standardize on incubation periods of two and four days, respectively, to facilitate plant control. With five-per cent salt agar two weeks' incubation was selected, and three weeks allowed for 10- and 15-per cent salt agar.

TABLE 1

Effect of Length of Incubation on Relative Counts at 20°C.(68°F.)
(Average of 11 samples)

Medium	Time in days				
	2	4	8	14	21
Nutrient agar	1	87	100	100	100
5% Salt agar	1	17	72	94	100
10% Salt agar	1	1	1	87	100
15% Salt agar	1	1	1	87	100

¹ Colonies undeveloped or ill defined

Effect of Salt Concentration on Plate Counts From Pickle: Only well-mixed representative samples of Wiltshire pickle taken before and after filtering were selected for quantitative analytical studies. At this stage the composition of different samples was essentially the same, the used pickle being rebuilt and mixed prior to filtering. The effect of salt concentration on plate counts from pickle is illustrated (Fig. 1), where the maximum logarithmic-average bacteria count (on 10-per cent salt agar) is taken to be 100. Increasing or decreasing the amount of salt in the medium reduced the number of bacteria obtained. On 15-per cent salt agar only 49 per cent of the maximum count was obtained and on five-per cent salt and nutrient agar the percentages developing were 59 and 8.8, respectively. On the latter medium at 37°C. only 1.6 per cent of the number of colonies developed.

While the bacteria counts on nutrient and salt agars showed a definite relationship, not only on the 50 selected samples but on more than 200 samples taken over a period of five years, it was observed that with improvements in plant sanitation the spread between nutri-

ent and 10-per cent salt agar counts became greater. This is believed due to lessened contamination (by non-halophilic organisms) of the sides going into pickle, since the trend was a reduction in nutrient agar counts rather than an increase in counts on 10-per cent salt
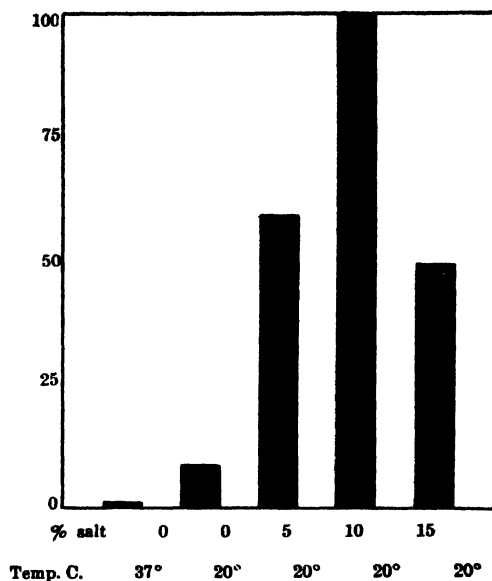
FIG. 1. Ratios of bacteria counts from Wiltshire pickle on nutrient and various salt agars. (Average from 50 samples, saline dilutions with salt agar.)

agar. The increasing difference is shown (Table 2), where the ratios of logarithmic-average bacteria counts from samples taken in the late autumn of four consecutive years are given.

TABLE 2

Ratios of Bacteria Counts From Wiltshire Pickle in Four Consecutive Years

Year	Month	Number of samples	Nutrient agar 37°C.	Nutrient agar 20°C.	10% Salt agar 20°C.
1935	Nov.-Dec.	8	1.5	7.7	100
1936	Oct. -Nov.	8	1.3	7.9	100
1937	Oct. -Nov.	7	0.8	4.4	100
1938	Oct.	11	0.5	3.4	100

Bacteria Counts on Miscellaneous Media: Irregular results were obtained with liquid and semi-solid media inoculated in serial dilutions in duplicate. Contrary to general experience with dilution methods the probable numbers of bacteria (calculated from Mc-Crady's tables) were lower in all cases than plate counts on 10-per

cent salt agar. This abnormality may be subject to qualitative explanation, for on 10-per cent salt agar the flora consists largely of obligate halophilic microörganisms which grow very indifferently if at all in liquid media.

In comparison with salt agar, greater counts were obtained on laboratory prepared pork infusion and lesser counts on the desiccated product (Table 3).

TABLE 3

Ratios of Bacteria Counts From 16 Samples of Wiltshire Pickle

Plating medium	Ratio
10% Salt agar	100
10% Salt agar + .1% KNO₃	126
10% Salt, pork agar (lab.)	150
10% Salt, pork agar + .1% KNO₃	155
10% Salt, pork agar (des.)	65
10% Salt, pork agar + .1% KNO₃	118

The addition of potassium nitrate to these and other media introduced marked variations in bacteria counts. Generally, but not always, significant increases in colony development resulted. Potassium nitrite was inhibitory, 90 per cent of those colonies capable of developing on 10-per cent salt agar being inhibited by concentrations greater than .4 per cent in the medium. Bacteria counts on 50-per cent pickle-infusion agar were in all cases lower than on 10-per cent salt agar.

Effect of Salinity of Dilution on Bacteria Counts: For quantitative analysis "square" Pyrex bottles containing 90 ml. of diluent were employed. In preliminary analyses no significant differences in bacteria counts were obtained when tap water or physiological saline dilutions were used. When higher salt concentrations were tested, however, it was apparent that the salinity of the dilution had a marked effect upon the counts obtained on high salt media. The change in osmotic pressure involved in the transfer from pickle containing approximately 30 per cent (wt./vol.) of sodium chloride to physiological saline renders incapable of developing many cells otherwise viable on salt agar. This effect is illustrated (Fig. 2 and Table 4).

TABLE 4

Average Bacteria Counts From Seven Samples of Wiltshire Pickle With Tap Water and 10-Per Cent Saline Dilutions

Dilution system	Medium	
	Nutrient agar	10% Salt agar
Tap water	41,500	27,600
10% Saline	42,300	941,400
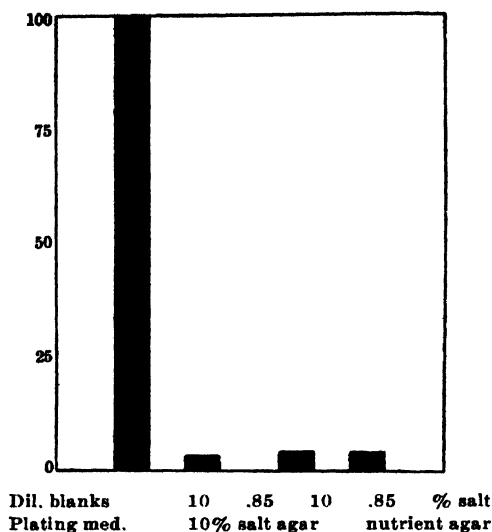
FIG. 2. Ratios of bacteria counts from Wiltshire pickle on nutrient and 10-per cent salt agar with physiological and 10-per cent saline dilutions. (Average from seven samples.)

The lethal effect of tap water or physiological saline dilutions upon certain bacteria was repeatedly observed in analyses of Wiltshire pickle and appeared to be instantaneous and little affected by the time the cells were exposed to the diluent as shown (Table 5).

TABLE 5

Effect of Duration of Exposure to Tap-Water Dilutions on Salt-Agar Counts From Pickle

Plating medium	Dilution	Time	Bacteria count
		min.	
10% Salt agar	10% Saline	15	2,200,000
	Tap water	15	50,000
	Tap water	1¾	50,200
	Tap water	1¼	59,200
	Tap water	¾	55,200

Likewise the effect was observed whether tap water was used for all dilutions or only in part (Table 6).

The direct relation between salinity of dilutions and bacteria count on 10-per cent salt agar is shown (Fig. 3), where the ratios of logarithmic-average bacteria counts from four samples of Wiltshire pickle are plotted against the salt concentration of dilutions. It will be noted that as the salinity decreased from 10 per cent the lethal

effect became greater and was greatest from 0 to four per cent. No increases were noted when sterile pickle or concentrations greater than 10-per cent saline were used for diluting samples. While most of the experimental work centered on pickle bacteria developing on 10-per cent salt agar, the effect was observed on five-per cent salt agar and

TABLE 6

Effect of Using 10 Per Cent Saline Dilutions in Part on Salt-Agar Counts From Pickle

Medium	Dilution system	Bacteria count	
		Test 1	Test 2
10% Salt agar	10% Saline all dilutions	6,000,000	252,000
	10% Saline first dilution only	320,000	11,500
	Tap water all dilutions	405,000	13,000

all higher concentrations, and in platings from slime on mature Wiltshire sides as well as from the curing pickle. It would appear that those organisms inhibited by non-saline dilutions are halophilic in
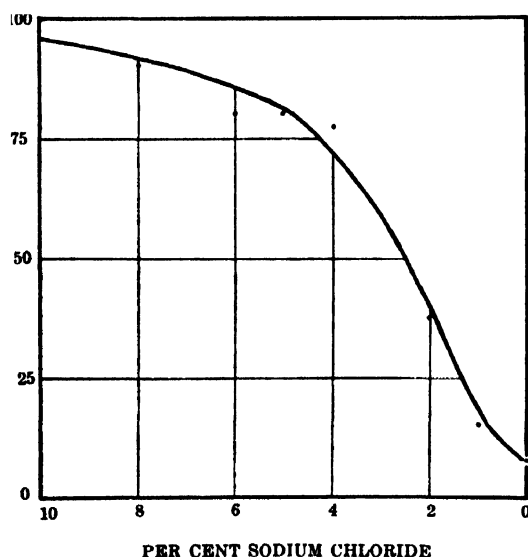
FIG. 3. Ratios of bacteria counts from Wiltshire pickle on 10-per cent salt agar according to salinity of dilution. (Average from four samples.)

nature rather than salt tolerant, for the percentage of halophilic organisms in pickle capable of developing on nutrient agar is in line with the relation of the reduced count on 10-per cent salt agar and the normal count on nutrient agar (Fig. 4).
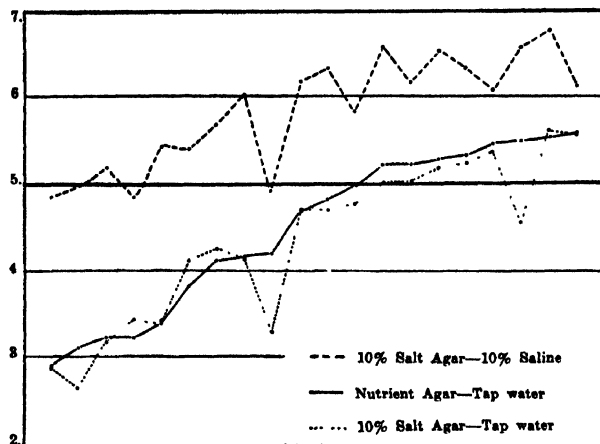
FIG. 4. Logarithmic-average bacteria counts from 20 samples of Wiltshire pickle on nutrient and 10- per cent salt agars with saline and tap-water dilutions.

DISCUSSION

The function of salt lies in its selective and inhibitive action towards certain types of bacteria. This action is most apparent in the study of bacteria developing on various salt media. The correlation between bacteria counts on nutrient and salt agars indicates the stability of an established pickle flora and may serve as an index of plant sanitation. It is generally believed that the role of organisms in the pickle is directly concerned with the reduction of nitrates and the little-understood alterations which differentiate a cured product from one merely salted. From the results obtained in this laboratory there is little evidence to show that bacteria are active in the pickle solution, for the maximum counts were obtained on media containing less than half the concentration of salt present in the pickle, and in liquid media of high-salt concentrations growth is negligible. It would appear that bacterial activity is confined largely to the immediate proximity of the side and even within the membranes of the side itself, for it is believed that here in the zone of salt transfer concentrations most suitable for the development of the pickle flora are to be found.

From the results obtained in varying the salinity of dilutions it would appear that there are extreme variations in the vitality of organisms in pickle, since over 90 per cent of the bacteria capable of developing on 10-per cent salt agar are destroyed on transfer to non-saline dilutions. According to Garrard and Lochhead (1939) certain substances are present in pickle which lessen the toxic effect of high-salt concentrations. Thus it is possible for certain non-salt-

tolerant organisms contaminating meat prior to curing to survive the pickling process and contribute to later storage defects. Pickle then may be regarded as containing not only obligate halophilic and salt-tolerant organisms, but non-salt-tolerant forms which may become active only after removal of sides from the high-salt environment of pickle. Thus the value of pickle analysis lies not only in the correlation of bacterial density with changes in the pickle but also with bacterial changes subsequent to curing as well as with pre-curing contamination. The existence of groups of diverse salt relationships in pickle necessitates the use of more than one medium for quantitative bacteriological analysis. A high bacteria count on nutrient agar or media containing less than five per cent of salt is apparently undesirable in pickle or at any stage in the cure, but there appears to be no great objection to moderate counts on 10-per cent salt agar.

In plant practice the bacterial content of curing pickle is limited by sanitary precautions and reduced by heating, skimming, settling, and filtering. The three great factors controlling bacteria in the pickle are temperature, salinity of pickle, and duration of the cure.

CONCLUSIONS

Experimental studies on Wiltshire pickle have shown that the rate of colony development and the total number of colonies varied with the salt concentration of the medium. Incubation of plates at 20°C.(68°F.) resulted in greater counts than at 37 or 5°C.(98.6 or 41°F.), and at 20°C. maximum bacteria counts were obtained on 10-per cent salt agar. Bacteria counts on nutrient, 5-, 10-, and 15-per cent salt agars showed a definite relation to each other on over 200 samples taken over five years. The ratio between nutrient and 10-per cent salt agar counts widened slightly, however, with increased plant sanitation.

Modifications of saline media including liquid and semi-solid dilution counts revealed fewer organisms than salt agar, and the addition of nitrite proved inhibitory. When nitrate was added to salt agar, irregular and unpredictable increases in plate counts were observed. Pickle infusion agar proved to be a poor medium.

To obtain maximum counts on salt media, saline dilutions were found to be essential, for over 90 per cent inhibition on 10-per cent salt agar resulted when physiological or tap-water dilutions were employed. The reduced counts on the salt agar were of the same order as counts obtained on nutrient agar and point to the elimination of a specific group of halophilic bacteria.

The five years' study of methods of analysis for Wiltshire pickle has shown that the flora is heterogeneous and requires at least two

distinct media for its enumeration, i.e., nutrient agar to indicate the degree of contamination and 10-per cent salt agar to reveal the natural or halophilic flora.

ACKNOWLEDGMENT

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CANADIAN WILTSHIRE BACON

I. OUTLINE OF INVESTIGATION AND METHODS[1]

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W. H. White[2]

Abstract

This paper introduces an investigation involving bacterial, chemical, and physical measurements on bacon and pickle from 22 packing plants. The objects were to determine the over-all variation occurring in practice, the general sources of the variation, and the specific effect of certain curing practices. Methods employed for shipping the samples, sampling, and analyses are described.

It was found that small samples of curing pickle change slightly in nitrite and bacterial content during shipping periods approaching a week's duration, but that these changes are of negligible importance from a practical standpoint if the pickles are kept at temperatures near the freezing point.

Introduction

During recent years the export of bacon as Wiltshire sides* from Canada to Great Britain has assumed considerable importance in Canadian agricultural economy. As a result, investigation of the many problems associated with the manufacture and export of this type of bacon has become increasingly necessary. The present paper, introductory to a series forthcoming from these laboratories, outlines the scope of an investigation into a number of these problems, and describes the methods used for transporting the experimental sides and pickle from the packing plants to the laboratory, and the sampling and analytical methods employed.

The packing plants engaged in the export bacon trade in Canada are widely distributed and the period required for transporting the product naturally varies considerably. A survey of the methods used for making Wiltshire sides showed that, although the individual plants adhered quite closely to their own particular method, there was considerable variability in the practices

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* *For a general description of Wiltshire sides, method of curing, and typical compositions of bacon and pickle, the reader is referred to the publications of the Food Investigation Board, London, England (1–4).*

adopted in different plants. This survey, however, gave little indication of the extent to which these variations affected the composition and quality of the bacon. Consequently, the present investigation was undertaken to determine: the over-all variation of the bacon with respect to the several measurements; the main general sources of variation; and more specific information on the effect of certain curing practices.

The over-all variation of Canadian bacon with respect to an individual property may be divided into: differences between sides from the same plant; differences between sides from different plants; and differences due to age from curing. The differences between sides from the same plant represent the combined effect of inherent differences in the curing quality of different carcasses and slight variations in the treatment each carcass may receive from the time of slaughter to the baling of the finished product. The difference between sides from different plants includes the sources of variation recognized above within factories, and also the additional effect of the different practices and pickle compositions used in different establishments.

Although a large number of factors may affect the composition and quality of bacon, particular attention has been given to the effect of the number of "stitches" (injections) per side used in pumping, the number of days in cure, and the composition of the pickles used. This approach to the problem was favoured, as curing is fundamental to bacon production. The particular factors mentioned above are apparently considered to be the most important, since each plant adheres closely to its chosen practice respecting them. Again, if necessary to improve quality, these factors could be modified promptly and inexpensively.

The relation between pickle and product is a function of the scale on which the curing practice is conducted, as shown by the fact that small pieces of pork "cured" in the laboratory (7) may have a salt content more than twice that of the factory product. If the results of investigations into the relations between pickle and product are to be applied in practice, it is essential that the materials studied be typical of those used or produced on a commercial scale. The practices followed in the 22 plants represented in this study varied sufficiently to give considerable information on the effect of pickle composition and other factors on the quality of the bacon.

It was necessary to employ statistical methods to interpret the large body of data obtained, while retaining certain confidential details. For details of the statistical methods used in this and subsequent papers the reader is referred to Snedecor (8).

Method of Shipping Samples

Both pickle and bacon are subject to chemical and bacteriological changes if held at ordinary temperatures, and special arrangements had therefore to be made for transferring these materials relatively unchanged from the plants to the laboratory. The methods employed are described below.

BACON

The period of transport from the plants to the port of Montreal varies from less than one to six days. To minimize abnormal changes during transport, the experimental sides were shipped with regular export shipments in a refrigerator car to the docks at Montreal. Immediately a car was opened, the samples were transferred to a room at 0 to 2° C. When the entire shipment of samples had been accumulated it was transferred to the laboratories by refrigerated truck.

PICKLE

Effect of Age and Temperature on Composition

Before undertaking these studies it was necessary to determine the conditions under which pickle may be shipped for periods of about five days duration without significant change in its chemical composition or bacterial content. In some preliminary experiments tank pickles, taken at the beginning, middle, and end of cure, were stored at 1.1 or 4.5° C. and at 25° C. for periods of about a week, in small sealed jars under aerobic conditions, since air was almost certain to be present in the test samples. Analytical results indicated that the chloride and nitrate content remained constant, while the nitrite content and bacterial numbers showed greater variation from day to day than could be accounted for by experimental error. This led to a more extensive study of the changes in nitrite content and bacterial numbers of pickles obtained from one plant at various stages of cure.

The changes in the nitrite content of five of the above pickles, representing the several types of change observed during storage at 25 and 4.5° C. or lower, are shown in Fig. 1, and the changes in the bacterial content in Fig. 2. The points in these figures represent the means of the determinations made at each sampling, and the ordinate of the cross-hatched section the difference necessary for statistical significance computed from the standard error of the replicated tests.

The nitrite content was determined colorimetrically by a procedure already described (9). The curves on the left-hand side of Fig. 1 were plotted from values obtained by comparing the colour developed in the sample with that of a standard solution of nitrite in a visual colorimeter. Those on the right-hand side were obtained with a photoelectric colorimeter, which yielded more precise results as shown by the smaller necessary difference.

Bacterial numbers were determined on nutrient agar and on 10% salt agar with incubation at 20° C. In Fig. 2 the ordinate represents the changes from the initial value during storage, these changes being expressed as the change in the logarithm of the bacterial number per ml. of pickle. The number of organisms observed on the medium containing 10% salt was much higher than that observed on salt-free media. The actual number present was not of particular interest in this study but will be discussed more fully in later papers of this series.
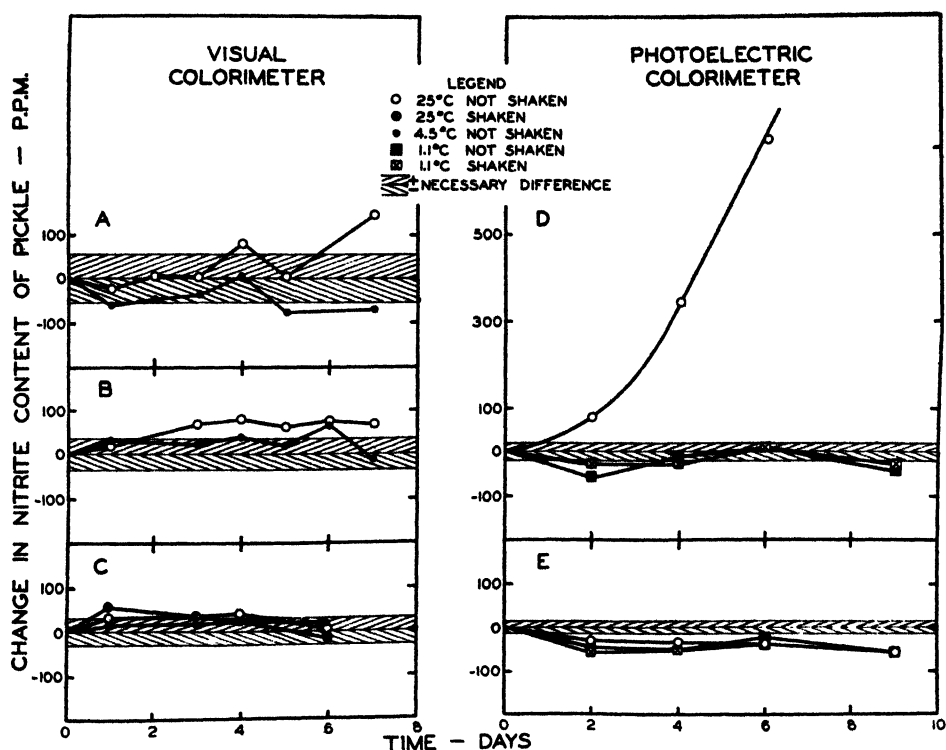
FIG. 1.   *Changes in nitrite content of pickle during storage at 25° C. and 4.5° C. or lower.*

The curves in Fig. 1 show that storage temperature had little effect on the nitrite content of certain pickles (*C* and *E*), while others show a small (*A* and *B*), or decided (*D*), increase in nitrite content during storage at room temperature.   Fig. 2 shows that the bacterial number observed on a salt-free medium remains relatively constant during storage at 4.5° C. or lower, but decreases during storage at 25° C.   The counts observed on a medium containing 10% salt vary with time, but there is no consistent effect of temperature.   These results showed definitely that the changes in nitrite content, and bacterial counts by certain methods, are reduced by keeping the pickle at a temperature of 4.5° C. or lower.   Methods for maintaining these low temperatures during transport for periods up to five days will be described in the next section.

The results obtained with pickles *C*, *D*, and *E* (Fig. 1) and *D* and *E* (Fig. 2) indicate that shaking, comparable with that occurring in transport, had no appreciable effect on the nitrite content or bacterial numbers.

It is evident from Figs. 1 and 2 that even when the pickles are kept at temperatures of 4.5° C. or lower, the changes in nitrite content and bacterial numbers during storage may exceed the "necessary difference", or the day-to-day variation attributable to experimental error.   The majority of these changes must therefore represent real alterations in composition.   These
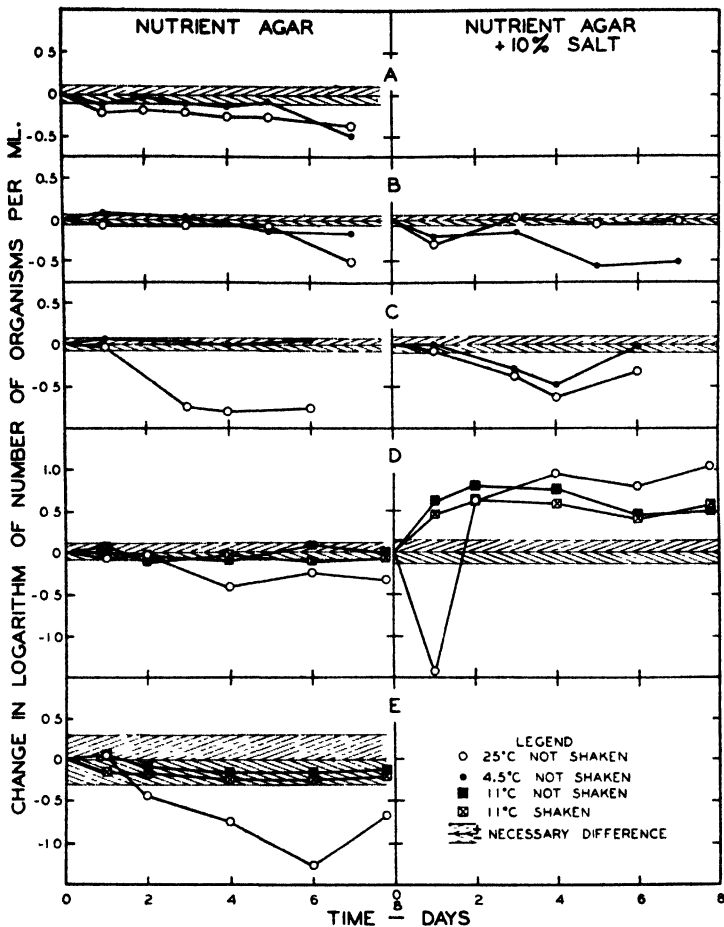
Fig. 2.   Changes in bacterial numbers in pickle during storage at 25° C. and + 5° C. or lower.

changes appear to be of two types: those showing a maximum or minimum during the storage period as shown by pickles A and B in Fig. 1, and B and C (10% salt medium) in Fig. 2; and those showing an increase or decrease only as shown by pickle E in Figs. 1 and 2.   The analyses on these and other pickles failed to show any definite relation between the type of change and the stage of curing, i.e., whether the pickle was taken at the beginning, middle, or end of cure.   Likewise the changes appear to be relatively independent of the original nitrite or bacterial content.   It seems probable that some of the rather sudden changes in composition may be related to such phenomena as protein precipitation.   It was therefore concluded that although pickles stored at low temperatures may show small but definite changes in composition, these changes depend on unknown properties of the individual pickle and are consequently unpredictable.   Ingram's (5) conclusion that tank pickles may be shipped for periods up to 10 days at temperatures below 10° C.

without appreciable change in their nitrite content does not appear to apply generally.

The differential changes in different pickles during transport must therefore be recognized as an additional source of error, to be combined with the sampling and experimental errors. By obtaining two samples of pickle from each plant at different times, it was possible to compute an over-all estimate of the experimental error, errors due to differential changes during transport, and the error of sampling the tank. The significance of differences between the composition of pickles from different plants could therefore be assessed by comparison with the variance within plants at different times. It is possible that this latter value yields an exaggerated estimate of the error attributable to sampling, changes, etc., since it is almost certain that the composition of the original pickle from an individual plant varies, at least slightly, from time to time.

Although a decrease in the bacterial content of pickle, as indicated by growth on nutrient agar at 20° C., appears to be associated with an increase in nitrite content during storage at 25° C. in these experiments, little significance can be attached to this observation. The small containers used were by no means typical of the storage tanks employed in practice, and the experiments were not conducted on a sufficiently extensive scale to permit definite conclusions.

Containers for Maintaining Low Temperature During Transit

The results of the previous section showed the necessity for using containers that would maintain the pickles in the vicinity of the freezing point during shipment to the laboratories, i.e., for periods up to four or five days' duration.

Four types of containers were tested: (i) a common "thermos" bottle of half-pint capacity; (ii) an insulated commercial isothermal jug of one gallon capacity containing a half-pint jar of pickle and 7 lb. of ice; (iii) a gallon pail insulated with $1\frac{1}{2}$ to 2 in. of cork and containing $4\frac{1}{2}$ lb. of ice in addition to the pickle jar; and (iv) a 2-gal. can insulated with 2 to $2\frac{1}{2}$ in. of wool felt and containing 15 lb. of ice. Typical results given in Table I show that the last container only was satisfactory, and this type was used throughout the investigation. The majority of the experimental samples were received at 0° C., a few at 5° C., and only an occasional one at 10° C. or higher.

Method of Sampling

BACON

The 44 sides of Wiltshire bacon, submitted by the 22 plants, were sampled for analysis three times: (i) on receipt at the laboratories, representative of their condition when shipped from Montreal; (ii) after 10 days' storage at 1.1° C., representative of their condition on arrival in London, England; and (iii) after smoking for 14 hr. at 43 to 46° C., which yielded material approximately representative of that in the British retail store. English practice favours smoking periods of 36 to 48 hr. at relatively low temperatures

TABLE I

TEMPERATURE IN °C. OF PICKLE KEPT IN CONTAINERS OF VARIOUS TYPES

Period of exposure at 25° C., days	"Thermos" bottle 1 pint capacity	Insulated commercial isothermal jug, containing ½ pint pickle + 7 lb. ice	Gallon pail insulated with 1½ – 2 in. cork dust, containing ½ pint pickle + 4½ lb. ice	2-gal. can insulated with 2 in. wool felt, containing ½ – 1 pint pickle + 15 lb. ice
0	2.3	0.2	0.0	0.0
1	12 5	1.1	0.0	0.0
2	17.8	16 7	1.0	0 0
3	21.0	—	11.1	0 0
4	22.5	—	19 7	0 0
5	—	— —	—	12.5

compared with Canadian methods. (In these studies the ham was the only portion of the side to be smoked. For this and other reasons the smoking period was reduced.)

On receipt at the laboratory the sides were placed in a room at 4.5° C. and all sampling was done at this temperature. The sides were unwrapped and samples for determining the bacterial number taken first. After removal of material for the chloride, nitrate, nitrite, moisture, pH, colour, colour stability, and tenderness determinations, and measurement of the oxidation-reduction potential of the whole meat in the ham, the sides were rebaled and transferred to a room at 1.1° C. After 10 days' storage the bales were again opened and sampled for these determinations, with the exception of tenderness measurements. This completed the sampling for bacterial numbers, chloride, pH, and oxidation-reduction determinations, but the ham from each side was smoked and again sampled for the other determinations.

Samples were also taken from the sides for studies on salt distribution, the effect of heat treatments comparable with smoking, and the fats. The samples used for these experiments will be described in the papers reporting the results.

The exact position from which the samples were taken and certain details of sampling can best be described by reference to Fig. 3. Samples of the pleural membrane over the second and third, and over the ninth and tenth ribs were removed for making bacterial counts at each sampling. At the first sampling the fourth and fifth ribs were removed without contamination of the pleural membrane and stored separately in an atmosphere of 95% relative humidity at 1.1° C. for a longer period than the sides were stored, to determine the surface bacterial number when visible slime became evident.

Material for the other determinations was obtained after the ham was removed, as indicated in Fig. 3. Samples for chemical analysis and the pH measurements were obtained by cutting a slice from the ham, while a triangular section removed from the side provided material for colour, colour stability,
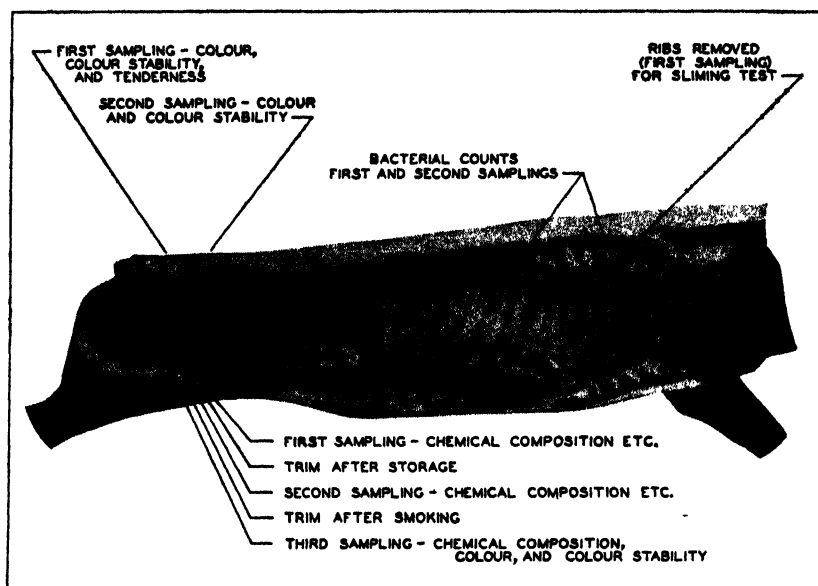
FIG. 3. *Portions of Wiltshire side used for determining bacterial counts, chemical composition, and physical properties at each sampling.*

and tenderness measurements. Oxidation-reduction potential was obtained by inserting a small pointed platinum electrode into the ham after removal of the slice for chemical analysis.

After storage the cut surface of ham was trimmed and another slice removed for analysis. At the same time a piece was removed from the side for colour and colour stability measurements. Oxidation-reduction potentials were again taken as described above. After smoking the ham was trimmed and another slice removed for both the physical and chemical measurements.

There are certain advantages and disadvantages in this more or less systematic method of sampling. Apart from certain practical limitations, it seemed desirable, since this study was concerned primarily with variations within and among plants, to take the samples from all the sides, at a given sampling, from comparable regions. This should permit more precise comparisons between the compositions of the same portion of different sides than if the samples had been taken at random from the whole side, or a preselected part of it. This procedure, however, sacrifices precision in determining the effect of ageing, for although the portions taken from each side at a given sampling are comparable, those taken at different samplings differ systematically in both time and position. In making the statistical analyses it is possible to determine the variance attributable to "between samplings" but it cannot be stated with assurance whether such differences represent a real change with time, or a difference between positions that existed originally. This subject will be discussed further in relation to the results obtained.

The slices of ham taken at each sampling for chemical analysis were immediately trimmed free of fat, bone, and connective tissue. Three small samples representing the outside, centre, and inside of the slice were removed for a study of salt distribution to be described in a later paper. The remainder of the lean meat was ground and thoroughly mixed by passing it through a food chopper several times. The pH of this ground material was immediately determined with a glass electrode, after which it was placed in a moisture-tight sample bottle, frozen in a room at $-29°$ C. and held at this temperature until required for analysis. Storage in the frozen state usually did not exceed a few days. The samples were thawed by placing them in a room at $4.5°$ C. for a few hours. They were then mixed thoroughly and portions for analysis weighed out in the same cold room. The sample residues were refrozen immediately to preserve the material for any determinations that required to be repeated. Extreme care was necessary at this stage since ground material kept at $4.5°$ C. for more than a few hours showed a significant increase in nitrite content, and this change was accelerated by exposure to higher temperatures.

All the above measurements were objective, and no attempt was made to estimate subjective qualities such as flavour. A special study of this important attribute of quality has been undertaken.

PICKLE

Two distinct pickles are used for curing Wiltshire bacon. One of these, designated "pump" pickle, is injected into the sides before they are placed in the curing vats, while the other, designated "tank" pickle, is used to cover the sides. Tank pickle suffers progressive changes in composition during the curing period. In order to obtain some estimate of the magnitude of these changes a sample of the tank pickle was taken when the sides were put in to cure, and again when they were removed. These two samples are subsequently designated "cover" and "spent" pickle respectively. When an estimate of the composition of tank pickle was necessary for certain computations, this was taken as the average composition of the cover and spent pickle with respect to the constituent in question.

Corresponding samples of pump, cover, and spent pickles were obtained twice from each plant with an interval of about a month between samplings. The second set of samples was taken from the pickles used for curing the bacon from which the experimental sides were obtained. In view of the small difference observed between successive pickle samplings, the mean composition of the two pickles was used for studying the relations between pickle and product in order to reduce the obscuring effect of experimental errors. The results of certain determinations on the pickles were lost or excluded as a result of errors in sampling, failures in transport, or losses in the laboratory.

When the pickle samples were received they were unpacked and placed in a room at $0°$ C. until the analyses were complete. Samples for bacterial numbers were removed to sterile glassware, plated, and incubated imme-

diately. Sufficient material for the chloride, nitrate, nitrite, pH, and oxidation-reduction potential measurements was removed to an ordinary laboratory for analysis and these determinations were made at room temperature. As the proteins tend to precipitate from pickle at room temperature the samples for this determination were pipetted from the original sample at 0° C. The remainder of the sample was filtered and colour measurements made in a room at 4.5° C.

Methods of Analysis

The methods employed for making the bacterial counts on both bacon and pickle will be described in later papers reporting the results of the measurements. The procedures followed in determining chloride, nitrate, and nitrite have already been described (9).

The protein nitrogen content of the pickle was determined on suitable portions (usually 10 or 25 ml. depending on the protein nitrogen content) pipetted into Kjeldahl flasks in the cold room. Nitrate nitrogen was removed (6, p. 27) before the Kjeldahl digestion.

The moisture content of the bacon was determined on 2- to 3-gm. portions of the ground material, by drying in flat aluminium dishes to constant weight (16 to 24 hr.) at 100° C. Preliminary measurements at lower temperatures yielded the same results, within experimental error, on this relatively fat-free material, but a much longer drying period was required. Actually it was difficult to attain a truly constant weight at any temperature, but after drying for 16 to 24 hr. at 100° C. the loss of weight over an additional 4-hr. drying period was never more than the equivalent of 0.05% moisture. Differences of this magnitude were smaller than the error between duplicates, and considerably less than the over-all sampling error.

The pH and oxidation-reduction potential measurements were made with a Bechmann pH meter. The appropriate standard electrodes supplied with the instrument were used for the determinations on pickles. The pH measurements on bacon were made in a room at 4.5° C., using a large glass electrode and extension leads. Both the glass and calomel electrodes were forced into a portion of the ground sample. Readings were required to check within 0.05 pH at different positions in the sample. Standard buffer solutions were used for adjusting the instrument at this low temperature, and all reported values were corrected to 20° C.

The oxidation-reduction potential of the bacon was measured with a special platinum electrode consisting of a 60° platinum cone, $\frac{1}{4}$-in. diameter at the base, carried on a $\frac{1}{4}$-in. diameter bakelite tube. This electrode was inserted to a depth of 2 to $2\frac{1}{2}$ in. into the ham and the circuit completed by inserting the calomel electrode into a cut at the surface. The observed potentials were converted to Eh by correcting for the potential of the calomel electrode at 4.5° C. The instrument and electrodes used for the Eh measurements were checked with a buffer solution of known pH containing quinhydrone. Several difficulties were encountered in making the Eh measurements in both bacon

and pickle, and there was some uncertainty in the results obtained.   These
will be discussed more fully when the results are presented in a later paper.

The colour and tenderness measurements on the bacon were made by means
of instruments and methods already described (10, 11).   These observations
were made on slices or portions of whole meat, immediately after cutting, in
a room at 4 5° C.   Samples were chosen that were free from obvious streaks
of fat or connective tissue.   An estimate of colour stability was obtained
by repeating the measurements on these slices periodically during exposure to
air at a temperature of 10° C. and a relative humidity of 95%.   This con-
dition avoided serious drying of the samples, which has been shown to affect
the colour (12).   The results therefore indicate the stability of the colour to
atmospheric oxidation at a temperature commonly prevailing in the storage
chamber of a retail store.

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CANADIAN WILTSHIRE BACON

II. CHLORIDE, NITRATE, AND NITRITE CONTENT OF BACON AND PICKLE[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

Analysis of Wiltshire bacon produced in Canadian plants showed that different sides varied in their chloride, nitrate, and nitrite contents, the chloride content being the most uniform. Although the observed variations were statistically significant they do not necessarily affect the quality of the product. An analysis of variance showed that differences between the sides from different plants were the main general source of variation in the chloride and nitrate contents, while the differences between sides from the same plant were the main source of variation in nitrite content. This, and other evidence, indicates that the properties of the individual side affect its nitrite content to a considerable extent.

The variation in the composition of the pickles used in different plants was significantly greater than the variations within plants, although the chloride content was relatively uniform throughout. Other curing practices, such as the number of injections used for pumping a side and the time in cure, also varied between different plants. Statistical computations showed that the number of injections used for pumping was correlated with the chloride and nitrite content of the sides, while their nitrate content was correlated with the nitrate content of the pump pickle. Although these factors affected the composition of the product with respect to each constituent, the level of the correlation coefficients was rather low. It is therefore concluded that most of the observed variation in the bacon was contributed by other unmeasured factors, or by inherent differences between the carcasses.

The analysis of variance showed significant differences between the content of the three constituents at different samplings. The method of sampling, however, did not permit the true effect of ageing to be distinguished precisely from the effect of systematic differences in position, and the observed differences between samplings might possibly have been due entirely to the effect of position.

Introduction

Although the quality of bacon may be affected by many factors, relatively few of these can be closely controlled. Those that can be standardized and reproduced from time to time in a given plant include the composition of the pickles with respect to the several salts used, and certain of the curing processes. Different plants, however, use different pickle formulas and curing practices, and this, together with the fact that the composition of the product may depend on other factors that are not closely controlled, can give rise to certain variations in the product. This paper reports the chloride, nitrate, and nitrite contents of the bacon, the general sources of variation, and more specifically, the effect of pickle composition and certain curing practices.

An outline of this investigation and a description of the methods used have been reported in the first paper (5) of this series. Statistical methods (7) were employed to reduce the large body of results, and permit their interpretation in terms of the required information.

The general mean and the standard deviation of a single observation served to describe the composition and over-all variation of the bacon and pickle with respect to each of the constituents. The coefficient of variability was computed from these quantities to facilitate comparison of the relative variability of different constituents. The practical significance of the observed variations had naturally to be assessed from other considerations.

The principal sources of variation were determined by an analysis of variance. For bacon, the total variance could be subdivided into portions attributable to: average differences between sides from different plants; differences between sides within plants; average differences between samplings; and sampling and analytical error. Although all the analyses of the samples were made in duplicate, it was impossible, within the limits of available time and material, to obtain and analyse two independent pieces from each side at each sampling. In consequence the residual variance was used to estimate the combined sampling and analytical error.

The results obtained with pickle were analysed in the same way except that only two sources of variance could be recognized, namely, within and between plants. For reasons already given (5) the variance within plants had to be taken as an estimate of the sampling and other errors. This variance probably overestimates these errors, since the samples for each plant were obtained at different times, and consequently the observed differences include any real change in the composition of the pickle from time to time.

The specific effect of pickle composition, number of stitches per side used in pumping, and the number of days in cure, on the concentration of salts in the product, was determined by computing simple correlation coefficients between the quantity of a given constituent in the bacon, the number of stitches per side, the number of days in cure, and the quantity of the same constituent in the pump and tank pickles. In some instances it was possible to combine two of these quantities. For instance the amount of salt contributed by the pump pickle may reasonably be taken as the product of its salt content and the number of stitches used per side. This assumption appears to be valid, since results by Callow (3, pp. 65–70) indicate that the amount of pickle retained is proportional to the number of stitches. Nevertheless some uncertainty remains, since the pumping equipment and method in different plants may not inject the same amount of pickle per stitch, while the number of stitches in the ham portion analysed may not remain in a fixed proportion of the number per side. Similarly the product of the chloride content of the tank pickle and the number of days in cure were used for computing certain correlations.

Finally the composition of the bacon will be affected by the composition and method of application of both the pump and tank pickles. The relative

effect of these two phases of curing cannot be established directly from simple correlation coefficients, since certain factors in each phase may be associated. Thus a plant using a weak pump pickle may also use a weak tank pickle, or a plant accustomed to the production of a mild bacon may reduce both the number of stitches used in pumping and the number of days in cure. By computing partial correlation coefficients, however, it was possible to determine the effect of the pumping practices independent of tank curing practices and vice versa. These computations were made wherever the simple correlations were significant, in order to establish the observed relation independent of associated factors.

Chloride

The mean chloride content of the bacon and pickles from all plants, over all samplings, appears in Table I. The average chloride content of 3.9% and a maximum of 5.8% indicate that Canadian Wiltshire is milder, on the average, than British bacon, if published results (1, 2) are typical of British curing practice.

The chloride content of the pickles is expressed on a weight-volume percentage basis, i.e., grams of sodium chloride per 100 ml. of pickle, or approximately lb. per 10 gal. The figures in Table I show that the mean chloride content of pump pickle approaches saturation (about 31.7% on a weight-volume basis at cellar temperature). Tank pickle is somewhat less concen-

TABLE I

CHLORIDE CONTENT OF BACON AND PICKLE

(As sodium chloride)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	3.93	29 20	28.32	23.80
Standard deviation, %	1.05	1 71	2.67	1.93
Coefficient of variability	26.79	5 86	9.41	8.10

Analysis of Variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.206 ⎫	19	0.795	16	0 824	16	1.280
Between samplings	1	21.315** ⎭						
Between sides within plants	22	0.476*						
Between plants	21	2.652**	(18)	5.178**	15	13.815**	15	6.323**

* *Indicates 5% level of significance, in this and subsequent tables.*
** *Indicates 1% level of significance, in this and subsequent tables.*

trated initially and the chloride content naturally decreases during cure, cf., values for cover and spent pickles respectively. Although the greatest absolute variations in chloride content occur in cover pickle, the chloride content of the bacon is relatively more variable than that of the pickles.

The results of analyses of variance are given in the lower part of Table I. Those for bacon show that, on the average, both the variance between samplings from the same side and that between different sides from the same plant were significantly greater than the sampling and analytical errors. The variance between sides from different plants, however, was significantly greater than that between sides within plants. The significance of the variance between samplings will be discussed more fully in a later section. Although it must be recognized that sides cured at the same time in the same tank differ significantly in chloride content, it is obvious that the major portion of the variation is contributed by the different chloride contents of bacon from different plants.

The simple and partial correlation coefficients between the chloride content of the bacon and the chloride contents of the pump and tank (mean of cover and spent) pickles, the number of stitches per side used in pumping, and the number of days in cure, appear in Table II. The simple correlation coefficient between the chloride content of the bacon and the number of stitches used per side is the only one that exceeds the 5% level of significance. In fact, the coefficients between the chloride contents of the bacon and that of both pickles, although insignificant, are negative. This indicates that the chloride content of the bacon is not affected by the observed variations in the chloride content of the pickles used, but depends entirely on other factors.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE CONTENT OF PICKLE AND CHLORIDE CONTENT OF BACON

(As sodium chloride)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean chloride content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.45*
Days in cure	20	−0.33
Chloride in pump pickle (mean)	19	−0.36
Chloride in tank pickle (mean)	19	−0.10
Loss of chloride from tank pickle during cure (mean)	19	−0.33
Chloride in pump pickle (mean) × stitches per side	19	0.40
Chloride in tank pickle (mean) × days in cure	19	0.25
Chloride in pump pickle (mean) × stitches per side, independent of chloride in tank pickle (mean) × days in cure	18	0.41
Chloride in tank pickle (mean) × days in cure, independent of chloride in pump pickle (mean) × stitches per side	18	0.28

The simple and partial coefficients between the chloride in the product and such combined quantities as chloride in pump pickle times the number of stitches, and chloride in tank pickle times days in cure, were insignificant.

Although these results indicate that the number of stitches used for pumping a side is more important than any of the other factors studied, the level of the correlation coefficient, although significant, is so low that less than 20% of the observed variance in the chloride content of the bacon can be accounted for by variations in the number of stitches used.   In consequence the major portion of the observed variation in chloride content, including that between sides from the same plant, must be due to unmeasured quantities concerned with curing, or to inherent differences between carcasses.   Fortunately the over-all variation observed cannot be regarded as having a serious direct effect on the quality of the product, although it may be desirable to produce bacon of more uniform chloride content.

Nitrate

The mean nitrate content of the bacon and pickles, the standard deviation from these means, the coefficients of variability, and the results of an analysis of variance are given in Table III.   The nitrate contents of the pickles are, cn the average, comparable with those used in British practice (3, pp. 65–70) and would appear to be satisfactory.   The nitrate content of both the product and the pickles is much more variable than their chloride content, as shown by the coefficient of variability.   The relatively greater variation observed

TABLE III

NITRATE CONTENT OF BACON AND PICKLE

(As sodium nitrate)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	0 184	2 32	1 32	0 92
Standard deviation, %	0 220	2 469	0 923	0 644
Coefficient of variability	119	107	69 7	70 4

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D f.	Mean sq.
Sampling and analytical error	86	0.0125 ⎫	19	0 342	16	0.380	16	0 056
Between samplings	2	0.0534* ⎭						
Between sides within plants	22	0 0228						
Between plants	21	0.2212**	18	12 173**	15	1.354**	15	0.798**

in the nitrate content of the bacon and pump pickle, as compared with that of the cover and spent pickles, was contributed largely by one plant that used much more nitrate than the average in its pump pickle.   This practice had a direct effect on the nitrate content of the product.

The analysis of variance shows that there was a significant difference in the nitrate content between samplings.   This phase will be discussed later.   The difference between sides from the same plant did not exceed the analytical and sampling errors significantly.   The difference between both the bacons and pickles from different plants, however, was significantly greater than the observed differences within plants.

The simple and partial correlation coefficients between the nitrate content of the bacon, and the known factors in the curing practice appear in Table IV. These correlation coefficients were computed on two bases, namely, including and excluding the results obtained from the plant using high nitrate concentrations in its pump pickle.   By including the results from this plant, the correlation coefficients are based on the figures from which the results in Table III were computed.   By excluding the results from this plant it was felt that the correlation coefficients were more typical of the effect of the ordinary variations in nitrate content of the pickles.   As can be seen from the table, the exclusion of these results tends to reduce the degree of correlation between the nitrate content of the meat and quantities including the composition of the pump pickle, and to increase that with quantities involving the composition of the tank pickle.

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN NITRATE CONTENT OF PICKLE AND NITRATE CONTENT
OF BACON

(As sodium nitrate)

Quantities correlated	Simple correlation coefficients			
	All plants		Exclusion of one exceptional plant	
	D.f.	r	D.f.	r
Mean nitrate content of bacon over all sides and samplings with:				
Pump stitches per side	20	0.13	—	—
Days in cure	20	0.29	—	—
Nitrate in pump pickle (mean)	19	0.92**	18	0.48*
Nitrate in tank pickle (mean)	19	0.59**	18	0.62**
Loss of nitrate from tank pickle during cure (mean)	19	0.09	—	—
Nitrate in pump pickle (mean) independent of tank pickle (mean)	18	0.88**	17	0.29
Nitrate in tank pickle (mean) independent of pump pickle (mean)	18	0.11	17	0.51*
Nitrate in pump pickle (mean) × stitches per side	19	0.89**	18	0.63**
Nitrate in tank pickle (mean) × days in cure	19	0.55**	18	0.57**
Nitrate in pump pickle (mean) × stitches per side, independent of nitrate in tank pickle (mean) × days in cure	18	0.80**	17	0.48*
Nitrate in tank pickle (mean) × days in cure independent of nitrate in pump pickle (mean) × stitches per side	18	0.02	17	0.39

Considering the coefficients given in the last column of Table IV it is evident that the nitrate contents of both the pump and tank pickles affect the nitrate content of the sides. This was to be expected, since the variation in the nitrate content of the two pickles was proportionately far greater than the methods used for their application. The partial correlation coefficients between the nitrate content of the bacon and that of the pump pickle, independent of tank pickle and vice versa, indicate that the nitrate content of the tank pickle has a greater influence on the composition of the product than that of the pump pickle. Nevertheless, the combined quantities, representing the known values of the pumping and tank-curing practices respectively, both yielded simple correlation coefficients that were highly significant. This suggests that the number of stitches per side and the number of days in cure, as well as the nitrate content of the two pickles, have some effect on the nitrate content of the sides. Partial correlation studies indicate that the composition of the pump pickle and the stitches per side are more influential than the composition of the tank pickle and the number of days in cure.

It is concluded from these results that the nitrate content of the pump and tank pickles and the number of stitches used in pumping are the principal factors affecting the nitrate content of the sides. It seems probable that, if more uniform pickle compositions and pumping practices were used in all plants, the relative variability of the product with respect to nitrate would be reduced to about the same level as that reported for chloride (Table I).

Nitrite

The mean nitrite content of the bacon and pickles, the variation of each, and the main sources of this variation are shown in Table V. The bacon con-

TABLE V

NITRITE CONTENT OF BACON AND PICKLE

(As sodium nitrite)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, p.p.m.	26 3	303	536	482
Standard deviation, p.p.m.	24.1	366	376	293
Coefficient of variability	91.6	121	70 2	60.7

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	127.8	19	8,331	16	6,551	16	3,966
Between samplings	2	818.6**						
Between sides within plants	22	1,213**						
Between plants	21	1,743	18	266,669**	15	285,593**	15	172,802**

tained 26 p.p.m. of sodium nitrite on the average. This is well within the 200 p.p.m. of sodium nitrite permitted by the Canadian pure food regulations (6). Although the coefficient of variability shows, on the average, considerable variation in the nitrite content of different sides, none of the sides, at any of the samplings, had a nitrite content approaching the legal limits.

The composition of the pickles with respect to nitrite content appears to be quite typical (3, p. 65–70), although these pickles were analysed during the summer months when the bacterial activity, and consequently the nitrite contents, would probably be maximal. On the whole the nitrite content shows about the same relative variability as the nitrate content.

The analysis of variance for bacon indicates that the difference between samplings and between sides from the same plant is responsible for most of the variance. In spite of the highly significant variations in the nitrite content of the pickles used in the different plants, the variance between the nitrite content of the bacon from different plants was not significant. This result is partly accounted for by the relatively large variance between sides within plants, most of which was contributed by the sides received from three or four plants. Detailed inquiry and examination of the results showed no valid reason for excluding these sides. The present investigation therefore indicates that the difference between sides treated in the same way is the main source of variation in nitrite content. More extensive analyses now under way, however, indicate that there is a significant difference between the nitrite content of sides from different Canadian plants after smoking in England. This cannot be taken as contradicting the present findings, since it will be shown later that the conditions used for smoking bacon in England may result in a differential development of nitrite in different sides.

The correlation coefficients between the nitrite content of the bacon and the known curing practices appear in Table VI. The correlation between the nitrite content of meat and the number of stitches used for pumping a side is the only one that is significant. Since correlations between the nitrite content of the bacon and that of the pump pickle never approach the level of significance, it appears that the number of stitches used for pumping increases the nitrite content of the bacon indirectly, rather than by a direct contribution of nitrite to the sides. This hypothesis is supported by the highly significant partial correlation between the number of stitches per side independent of nitrite in pump pickle. The possible nature of such an indirect effect is obscure, but it may result from the introduction of air or bacteria into the meat during pumping. It will be shown in a later paper that the pump pickles contained a considerable number of aerobic bacteria.

If nitrites can be produced from nitrates either on or within the sides after curing, it is evident that the observed nitrite content of the sides reflects both the extent or rate of this reaction as well as that between nitrite and haemoglobin, or muscle proteins. An attempt was therefore made to determine which of the two reactions was the more important in determining the nitrite content of the bacon. This was done by computing the nitrate : nitrite

TABLE VI

COEFFICIENTS OF CORRELATION BETWEEN NITRITE CONTENT OF PICKLE AND NITRITE CONTENT
OF BACON

(As sodium nitrite)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean nitrite content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.57**
Days in cure	20	0.29
Nitrite in pump pickle (mean)	19	0.25
Nitrite in tank pickle (mean)	19	0.25
Loss of nitrite from tank pickle during cure (mean)	19	0.21
Nitrite in pump pickle (mean) independent of stitches per side	18	0.21
Pump stitches per side independent of nitrite in pump pickle (mean)	18	0.57**
Nitrite in pump pickle (mean) independent of nitrite in tank pickle (mean)	18	0.15
Nitrite in tank pickle (mean) independent of nitrite in pump pickle (mean)	18	0.15
Nitrite in pump pickle (mean) × stitches per side	19	0.34
Nitrite in tank pickle (mean) × days in cure	19	0.23
Nitrite in pump pickle (mean) × stitches per side, independent of nitrite in tank pickle (mean) × days in cure	18	0.27
Nitrite in tank pickle (mean) × days in cure, independent of nitrite in pump pickle (mean) × stitches per side	18	0.12

ratio in the pickles and bacon from the individual plants. It was felt that if this ratio was significantly lower in the bacon than in the pickle, production of nitrite in the sides would be indicated, while a higher ratio in the bacon would suggest that reactions favouring the disappearance of nitrite predominated. Moreover, the use of the ratio would tend to minimize the effect of systematic variations in the composition of the meat between the different positions.

These ratios were found to be extremely variable both in the bacon and in the pickle. When they were subjected to an analysis of variance, neither the difference between samplings, between sides from the same plant, or between sides from different plants were significantly greater than the residual variance attributable to sampling and analytical error. The nitrate: nitrite ratio in the tank pickle only showed significant differences among plants. In fact, the mean ratio for the bacon over all samplings and sides did not differ significantly from that of the pickle over all samplings and plants.

It was observed, however, that two sides from each of two plants had a very low nitrite content at the first sampling, and since the nitrate content was about average, the nitrate : nitrite ratio was exceedingly large. The nitrite content at the later samplings, however, had increased considerably, indicating the production of nitrite within these sides. Since the different

sides in this group showed considerable variation in nitrite content at all samplings it was necessary to test the significance of the observed differences. For doing this the nitrate : nitrite ratio was used in preference to the nitrite content, for reasons already given.

Since the mean nitrate : nitrite ratio of these four sides varied widely both between sides and samplings, with the variance approximately proportional to the mean, it was necessary to use the logarithm of these ratios in making the analysis of variance in order to provide valid tests (4) of significance. The results of this analysis appear in Table VII, from which it is evident that the differences between sides and samplings are both significantly greater than the residual variance.

TABLE VII

ANALYSIS OF VARIANCE OF SAMPLES HAVING LARGE
NITRATE : NITRITE RATIOS INITIALLY

Figures on basis of logarithms of ratios

Variance attributable to	Degrees freedom	Mean square
Between sides within times	3	2.01*
Between times (samplings)	2	2.62*
Residual	6	0.412

It is concluded from these results that sides having a high nitrate : nitrite ratio initially, i.e., low nitrite content, may increase in nitrite content during maturation and smoking. Furthermore, the difference between sides indicates that the extent of this increase depends on the properties of the individual sides. Whether the variable increases in the nitrite content of different sides were due to varying bacterial loads or to inherent differences in the carcasses themselves is not known. Since this behaviour was not observed over all samples it appears that under average conditions nitrite production does not occur to any significant extent in samples containing an average nitrite content initially.

Difference Between Samplings

Certain changes, termed maturation, occur in bacon after removal from cure. Although these are believed to be beneficial to the general quality and flavour of the product (3, pp. 70–72), their nature is obscure. It therefore seemed desirable to consider the changes that occurred in all of the individual constituents and properties measured. The results given in previous tables showed that the three constituents dealt with in this paper did differ significantly between samplings. However, as pointed out in the first paper (5), the difference between samplings includes the effect of systematic differences between the positions from which successive samples were taken as well as the true effect of ageing and smoking. Since certain constituents, such as chlorides, should not suffer any change with time, these can be used as reference substances for assessing the significance of observed differences in other constituents which might change, e.g., nitrites.

Table VIII shows the mean chloride, nitrate, and nitrite contents over all sides by samplings, the difference between these means, and the significance of the differences compared with the sampling and analytical error. All three constituents showed a significant increase between the first and second samplings. The chloride content was not determined at the third sampling. Between the second and third samplings, the nitrate content remained practically constant, and the nitrite content decreased significantly. The increased chloride content must represent the effect of position or chloride distribution, which will be discussed in a later paper. A similar conclusion must be reached for nitrate since there is no evidence that any appreciable quantity was converted to nitrite. Since the increase in nitrite between the first and second samplings is of the same order on a percentage basis as that observed with chloride and nitrate, it appears that this increase represents the effect of position rather than time. The decrease in nitrite between the second and third sampling may indicate a decrease in nitrite during smoking, since the nitrate content remains practically constant.

TABLE VIII

DIFFERENCE BETWEEN SAMPLINGS

Constituent	Mean by samplings			Difference between means		
	First (1)	Second (2)	Third (3)	1—2	2—3	1—3
Sodium chloride, %	3.43	4.42	—	0.985**	—	—
Sodium nitrate, %	0.144	0.204	0.205	0.060*	0.001	0.061*
Sodium nitrite, p.p.m.	22.6	31.1	25.2	8.42**	5.83*	2.59

A further attempt was made to determine the effect of age on nitrite content by computing simple correlation coefficients between the nitrite content and the age of the bacon at the three samplings independently, and over all analyses irregardless of samplings. These were found to be -0.04, -0.20, and -0.28, for the first, second, and third samplings respectively, and $+0.02$ over all samplings. None of these coefficients is statistically significant, although the negative sign within samplings indicates a decrease in nitrite with time. When the computation is made over all samplings, the effect of position intervenes, decreasing the correlation coefficient and changing its sign.

In conclusion there is no evidence to indicate that, on the average, a serious change occurs in the nitrite content of bacon during storage at $1.1°$ C., or smoking for 14 hr. at about $45°$ C. There is some indication that nitrite may be formed in sides having a low nitrite level on removal from cure. The difference between the nitrite contents of sides cured in the same plant suggests that much of the observed variability is due to some factor that is not closely controlled in commercial practice. This factor may be some inherent property of the individual sides, or differential bacterial contents.

Difference Between Bacon and Pickle from Different Plants

The results reported in earlier tables show that although the differences between sides from the same plant were generally significant, the differences between sides from different plants were usually greater. It is therefore of interest to determine how the concentrations of the several salts in the sides and pickles from the various plants are distributed around the general mean for each constituent.

Before preparing these frequency distributions, the difference necessary for statistical significance was computed for the variance between sides within plants for bacon, and from the variance within plants at different times for the pickles. These necessary differences were then used as the class interval in preparing the frequency distribution for each constituent. This method has the advantage of distributing the observed values over the number of classes that can be distinguished experimentally from one another, although individual results in adjacent classes may not differ significantly. It must be recognized, however, that a large number of classes will be distinguished if the material is variable with respect to the measurement in question, or if the variance between sides and pickles from a given plant is small, and vice versa.

The frequency distributions for the chloride, nitrate, and nitrite contents of the bacon and pickle appear in Fig. 1. Four classes can be distinguished in the bacon with respect to chloride content. Since the distribution around the mean is symmetrical, with only a few plants falling in the extremes, the

CLASS INTERVAL EQUALS NECESSARY DIFFERENCE
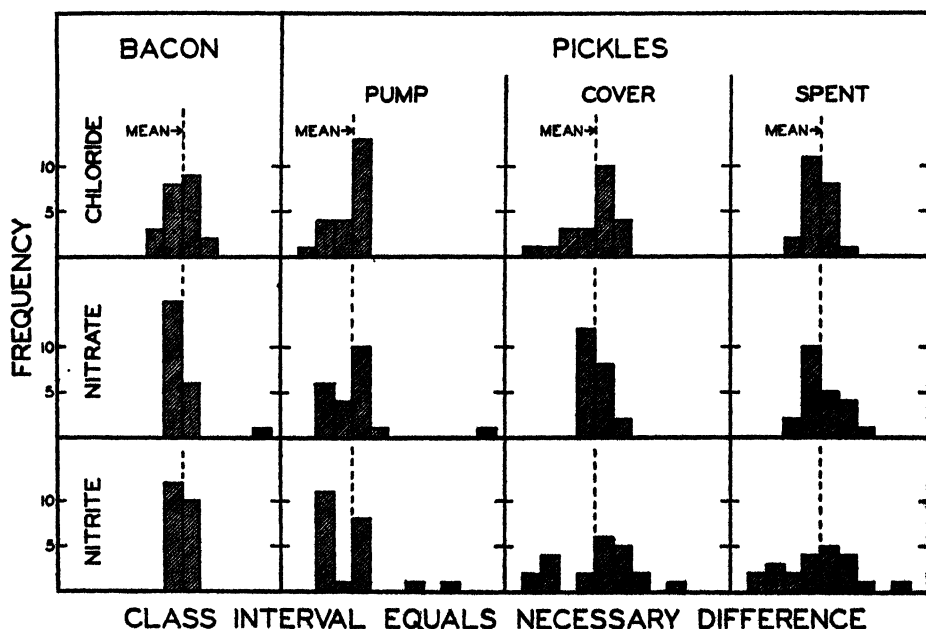
FIG. 1. *Frequency distributions of chloride, nitrate, and nitrite contents of bacon and pickle from different plants.*

chloride content of the product can be considered satisfactory. With the exception of the one plant using a high nitrate content in its pump pickle, only two classes can be distinguished with respect to the nitrate and nitrite contents of the bacon. This is due to the considerable difference observed between the content of these constituents in sides from the same plant.

The chloride content of the pump pickles falls into four classes, the distribution showing that the majority of the pickles are approximately saturated. Since the necessary difference for the pump and cover pickles was approximately the same, the more variable chloride content of the cover pickle is shown by a distribution over six classes. Only four classes of spent pickle could be distinguished.

The nitrate contents of the several pickles fall into three or five classes. Since the necessary differences were relatively large for this constituent, considerable variability is indicated. It has already been shown (Table IV) that the nitrate content of the pickles affects that of the bacon. It appears therefore that the use of pickles of more uniform nitrate content in the different plants would be desirable.

The nitrite content of the pickles was more variable than either the chloride or nitrate contents, since, in spite of the relatively large necessary differences, the values are scattered over about seven distinct classes. Although no direct relation could be demonstrated between the nitrite content of the pickle and bacon (Table VI), and in fact, there was some indication that the properties of the individual side determine its nitrite content, it would nevertheless seem desirable to standardize the nitrite content of the pickles used in the different plants as far as possible. In this connection it must be kept in mind that the combination of nitrite with the muscle pigments and proteins may occur differentially in different sides, and thus contribute to the observed variability.

In conclusion it should be pointed out that, although these results show statistically significant differences between the chloride, nitrate, and nitrite contents of bacon and pickle from different Canadian packing plants, it is not known that these variations seriously affect the final quality of the product.

Acknowledgments

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CANADIAN WILTSHIRE BACON

III. pH, OXIDATION-REDUCTION POTENTIAL, AND MISCELLANEOUS MEASUREMENTS ON BACON AND PICKLE[1]

By W. H. Cook[2] and A. E. Chadderton[3]

Abstract

The pH of bacon was relatively uniform, but it was possible to demonstrate statistically significant differences between sides cured in the same plants. Sides from different plants were however no more variable than those from the same plant. The results indicate that the pH of the bacon is affected by the pH of the pump pickle, decreases with the time in cure, and increases with the age from cure.

The absolute values of the Eh potentials observed in bacon were doubtful, but since the measurements indicated a statistically significant difference between sides from different plants it appears that this property may be a function of curing practice. Although the moisture content of bacon was relatively uniform, there was a significant difference between sides from different plants, and a significant loss of moisture during maturation and smoking.

The protein content of the tank pickle from different plants varied considerably, and probably reflects the effect of different handling practices. Nevertheless it was possible to demonstrate a direct relation between protein content and pH of the pickle.

Introduction

This paper constitutes one of a series covering an investigation of factory-cured Wiltshire bacon. An outline of the complete investigation and the methods employed were reported in the first paper (7). Of the many factors that may affect the quality of bacon, only a limited number can be controlled in commercial practice. For instance, the salt, nitrate, and nitrite contents of the pickle, and certain curing practices, such as the method of pumping sides, and the curing time, are ordinarily controlled and standardized within a given factory. This gives some control over the composition of the product with respect to the curing salts. These constituents and practices have been dealt with in an earlier paper (8). Other factors which are not controlled to any extent in either the pickle or the product, except indirectly, may also have some influence on the quality of the bacon. These include the pH and oxidation-reduction potential of the pickle and product, the protein content of the pickle, and the moisture content of the bacon. This paper deals with measurements of these properties in bacon and pickle from different packing plants.

Certain evidence (4, 5, 9) indicates that the pH of both the pork and the pickles may affect the final quality of the bacon with reference to its water and salt content, colour, and subsequent taint development. Indirect inform-

ation suggests that the oxidation-reduction potential may influence the colour. Brooks (2) has shown that, even in the absence of oxygen, the reaction between haemoglobin and nitrite yields a mixture of both nitrosohaemoglobin and methaemoglobin, and that the presence of a reducing agent is required to prevent the formation of the latter compound. Since methaemoglobin is not found to any extent in the interior of bacon, it follows that the tissues contain some reducing substance having an oxidation-reduction potential lower than that of haemoglobin-methaemoglobin (6).

Other results (3) indicate that the moisture content of the lean part of the bacon decreases as the salt content increases, even after due allowance has been made for the salt present. The protein content of the tank pickle may affect other properties, for example, rate of bacterial development and consequently nitrite production, and thus indirectly affect the quality of the final product. The results of these miscellaneous measurements are reported in this paper. The interrelations between the several constituents in, and properties of, the product will be discussed in a later article.

The detailed observations on the several properties were reduced by statistical treatment (12) and are presented in the same form as that used in an earlier paper (8).

Hydrogen Ion Concentration

The mean pH values over all samples of bacon and pickle, and their standard deviations on a single observation basis, appear in Table I. The results show that the pH of the bacon from the various sources was remarkably uniform, with a mean value of pH 5.74. In general, the pump pickles were

TABLE I

pH OF BACON AND PICKLE

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean	5.74	7.86	6.80	6.42
Standard deviation	0.14	0.58	0.67	0.21

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.0064	18	0.291	16	0.270	16	0.048
Between samplings	1	0 1023**						
Between sides within plants	22	0.0264**						
Between plants	21	0.0371	17	0 379	15	0.645*	15	0.040

* *Indicates 5% level of significance in this and subsequent tables.*
** *Indicates 1% level of significance in this and subsequent tables.*

alkaline, having a mean pH of 7.86. The tank pickle was slightly acid initially and became more acidic during cure. Even at the end of cure, however, it was still more alkaline than the sides. The variability in the pH of the pickles appears to be related to their protein content and buffer capacity, since spent pickle, having the highest protein content (Table V), is the least variable.

The results of an analysis of variance appear in the lower part of Table I. In spite of the small over-all variation in the pH of the bacon, the differences between samplings and between sides from the same plant were highly significant, while the differences between sides from different plants were not significantly greater than those observed within plants. Other investigations (4, 5, 11) have shown that there is considerable variability in the pH of pork. The findings suggest that the small variations in the pH observed in bacon may be due primarily to differences in the pH of the pork.

The difference between the pH of the pickles from different plants was significantly greater than the variations within plants for the cover pickle only. There was some evidence that there was a significant variation in the pH of the pump pickle in a given plant from time to time, but for reasons already given (7) it was impossible to distinguish precisely between variability originating from this source and that attributable to sampling and other errors.

Simple correlation coefficients were computed between the mean pH of the bacon over all samplings and the pH of the pickles and other known curing practices, as outlined in Table II. The three significant correlation coefficients obtained show that the pH of bacon is inversely associated with the days in cure and with the pH of the tank pickle × days in cure, and directly related to the pH of the pump pickle. It appears that the number of days in cure is primarily responsible for the significance of the combined quantity.

TABLE II

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN THE pH OF PICKLE
AND pH OF BACON

Quantities correlated	D.f.	r
Mean pH of bacon over all sides and samplings with:		
Pump stitches per side	20	0.00
Days in cure	20	−0.52*
pH of pump pickle (mean)	20	0.46*
pH of tank pickle (mean)	20	−0.09
pH of pump pickle (mean) independent of pH of tank pickle (mean)	19	0.49*
pH of tank pickle (mean) independent of pH of pump pickle (mean)	19	−0.20
pH of pump pickle (mean) independent of days in cure	19	0.32
Days in cure independent of pH of pump pickle (mean)	19	−0.41
pH of pump pickle (mean) × stitches per side	20	0.09
pH of tank pickle (mean) × days in cure	20	−0.50*

Partial correlative coefficients between the pH of the bacon and the pH of the pump pickle independent of days in cure, and days in cure independent of pH of pump pickle, were both insignificant. These results show that although both these factors influence the pH of the bacon, it cannot be said which is the more important.

Certain supplementary studies were therefore made to obtain more definite information. It was found that while the pH of pork was scarcely affected by immersion in pickles at pH 5.0 to 5.5 for periods of a day or two, the injection of relatively small quantities of acidic but unbuffered brines, comparable with pump pickle, had an immediate and permanent effect on the pH of the pork. This suggests that if it is desirable to modify the pH of pork by means of the pickle, acidification of the pump, rather than the tank pickle would be the more effective method.

It is known that the pH of pork varies considerably (4, 5, 11). Unpublished results indicate that under Canadian conditions it may range from pH 5.5 to 6.5. Since the pH of the bacon was 5.7 (Table I), it appears that the meat must have become more acidic during cure. This could scarcely have resulted directly from the injection or absorption of pickle, since the pickles were generally alkaline to pH 5.7. These considerations suggest that the extent of the reaction affecting the pH of the bacon is affected by the length of the curing period independent of the pH of the curing pickles.

Oxidation-reduction Potential

The methods employed for measuring the oxidation-reduction potential of both the bacon and pickle have already been described (7). These results were subject to some uncertainty for the following reasons: (i) the potentials observed in both bacon and pickle showed considerable "drift" with time, and although the readings were not accepted until equilibrium had apparently been established, it is probable that errors of the order of 5 to 10 mv. occurred in some samples; (ii) since the pickle samples were exposed to air during transport, sampling, and measurement, it seems likely that the observed potentials were somewhat higher than those occurring in the curing tanks; (iii) for bacon two apparently identical electrodes gave, on the average, widely different absolute potentials.

In spite of these uncertainties the results are reported in Table III, since certain deductions are possible. The values reported for bacon were obtained with the No. 1 electrode, which gave a mean potential of about 24 mv. as compared with a value of about -121 mv. obtained with the No. 2 electrode on the same samples. The results obtained with the No. 1 electrode are preferred since it reproduced, from time to time, the correct potential in a quinhydrone-buffer solution more closely and consistently than the No. 2 electrode.

All three pickles were found to have about the same mean potential. It seems probable that the observed values represent the actual potentials in

TABLE III

OXIDATION-REDUCTION POTENTIAL (Eh) OF BACON AND PICKLE

Statistic	Bacon (Electrode No. 1)	Pickle		
		Pump	Cover	Spent
Mean, mv. (+)	23 7	338	328	328
Standard deviation, mv.	89.9	31.8	30 2	35 6

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	31	2,489 ⎫	18	975	16	554	16	628
Between samplings	1	9,025 ⎭						
Between sides within plants	22	1,636						
Between plants	21	22 422**	17	1,044	15	1,294	15	1,952*

the pickles as received, as they were reproducible, within the error attributable to "drift", with different electrodes. Since these samples had suffered exposure to air, however, there is some doubt that they represent material at all comparable with that existing in a curing tank.

In order to obtain information on this point, the oxidation-reduction potential was measured daily at four positions in a tank during cure. The four positions represented two depths, 4 in. and 3 ft. 10 in. below the surface, at two places in the tank. These readings varied considerably with position, depth, and time, and further information is required before a definite statement can be made as to the effect of these variables. It is sufficient to point out that the maximum, minimum, and mean values observed were 245, − 189, and 86.2 mv. respectively. Since these values are considerably lower than those reported for tank pickle in Table III, it would appear that a satisfactory estimate of the oxidation-reduction potential can only be obtained on samples that have not suffered undue exposure to air.

Although the observed potentials in the main series of experiments may not be absolute, the results of an analysis of variance are of interest. The difference between plants, for bacon and spent pickles, was the only statistically significant source of variance, distinguishable from experimental error. In this respect both the electrodes used in bacon yielded similar results. It appears from this that the oxidation-reduction potential of bacon is dependent on the handling and curing practices followed in a particular plant.

Because of the uncertainty of the results, only a few correlation coefficients were computed. The coefficient between the Eh of bacon (No. 1 electrode) and that of the tank pickle (mean of cover and spent) was insignificant ($r = 0.25$ for 19 degrees of freedom). Although the Eh of both spent pickle

and bacon differed significantly between plants, they were not significantly correlated ($r = 0.11$).

The results presented in an earlier paper (8) showed that the nitrite content of the bacon was related to the number of stitches used in pumping, independent of the nitrite content of the pump pickle. It was felt that this might be the result of the introduction of air or bacteria with the pump pickle. Since the introduction of air might affect the oxidation-reduction potential of the bacon, the correlation coefficient between this quantity and the number of stitches per side was computed. The insignificant value ($r = 0.03$) was obtained. This does not necessarily disprove the above hypothesis, as the measurements on the bacon were made a considerable time after the pumping operation.

Moisture Content of Bacon

The moisture content of the bacon was determined at all three samplings. The results, including the analysis of variance, appear in Table IV. The mean moisture content over all sides and samplings was 71.38% with a relatively small variation. Nevertheless, an analysis of variance showed that the differences between samplings and between sides from different plants were highly significant. The loss of weight during storage and smoking doubtless accounts for the differences between samplings, a subject to be discussed further in a later section of this paper. The differences between the moisture content of the sides from different plants may be the result of the differences in salt content (3). These relations will be presented in a later paper.

TABLE IV

MOISTURE CONTENT OF BACON

Statistic	
Mean, %	71.38
Standard deviation, %	1.58
Coefficient of variability	2.22

Analysis of variance

Variance attributable to	D.f.	Mean sq.
Sampling and analytical error	86	0.621
Between samplings	2	93.968**
Between sides within plants	22	0.970
Between plants	21	3.100**

Protein Nitrogen Content of Pickle

The results of the Kjeldahl nitrogen determinations appear in Table V. Although expressed as the percentage of protein nitrogen this determination also includes lower compounds which yield ammonia on digestion. The mean protein nitrogen content of the pump pickle was 0.0008%. In some

instances, however, the samples contained almost as much as certain cover pickles. No statistical computations were made on the results obtained with pump pickles. The results in Table V show that the mean protein nitrogen content of cover pickle was about 0.09%, while that for spent pickle was 0.12%. The analysis of variance shows that the protein content of the pickles from different plants differs significantly, and probably reflects the effect of the different handling, treatment, or storage practice.

TABLE V

PROTEIN NITROGEN CONTENT OF PICKLE

Statistic	Cover	Spent
Mean, %	0.092	0.121
Standard deviation	0.049	0.040
Coefficient of variability	53.5	33.2

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error and variance between samplings	16	0.00032	16	0.00053
Between plants	15	0.00467**	15	0.00278**

Although the observed variations in tank pickle could be accounted for in this way, an attempt was made to determine whether the solubility of the protein was dependent on any other properties of the pickle. The computation of correlation coefficients between the protein content and the pH and salt content of the pickle yielded values of 0.63 (highly significant), and 0.25 (not significant), respectively. It is therefore concluded that, whereas the observed variations in the salt content of the pickle (8) have no effect, the solubility of the proteins increases with increase in pH within the range (Table I) experienced in practice. This latter finding is in general agreement with the results of other investigations (11).

Difference Between Samplings

It has already been pointed out (7) that the design of the experiment did not permit accurate estimation of the effect of time, as the observed difference between samplings included both systematic differences between the positions from which successive samples were taken, and the true effect of ageing. The results obtained with salt and nitrate (8) led to the conclusion that a systematic difference between positions did exist. In consequence the observed differences between the nitrite contents at the several samplings had to be attributed to the effect of position rather than time. The question therefore arises as to whether the observed changes in the pH and moisture content of the bacon

represent a real change with time or merely variation between different positions.

The mean pH and moisture content of the bacon at each sampling is given in Table VI. These results show that both the pH and moisture content decreased significantly between successive samplings. Since evaporation is to be expected during storage and smoking, the decrease in moisture content doubtless indicates a real effect of time rather than the influence of position.

TABLE VI

DIFFERENCES BETWEEN SAMPLINGS IN pH AND MOISTURE CONTENT OF BACON

Constituent or property	Mean by samplings			Remarks
	First	Second	Third	
pH	5.78	5.71	—	Differences between means highly significant
Moisture, %	72.83	71 39	69.91	Differences between any two means highly significant

The decrease in pH between samplings, although significant, is rather small, and might have resulted from the influence of position rather than a real change with time. Earlier results (Table II) indicate that the pH of bacon may be affected by the time in cure, and it is reasonable to believe that the pH might also be affected by the time from cure. The difference between the mean pH values at the different samples is not a satisfactory method of studying the effect of ageing, since the sides varied in age from 2 to 11 days at the time of the first sampling. Consequently, any change that takes place with time might reasonably have occurred to various extents in the sides, quite apart from the uncertainties arising from the effect of position.

In order to obtain more definite information on the effect of time, at various stages, on the pH of bacon, simple and partial correlation coefficients were computed between the pH of the bacon and the elapsed time before, during, and after cure. By making these computations separately for each sampling, the possible effect of differences between the positions from which successive samplings were taken was excluded. The results appear in Table VII.

Since the correlation coefficient between pH and days from slaughter to cure was not significant, it appears that the ordinary variations in cooling time have no effect on the acidity of the smoked bacon.

Since the pH of the pump pickle and the period in cure have been shown to affect the pH of bacon to some extent, it might be that the length of holding period prior to cure had an effect on the pH of pork which was subsequently altered by other factors. However, the results of investigations on rabbit and poultry muscle (1, 10), and some unpublished results on pork, indicate that the ultimate pH of muscle tissue is attained well within the shortest cooling period used in practice.

TABLE VII

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN pH OF BACON AND TIME
IN CURE AND AGE FROM CURE

Quantities correlated	D.f.	r
pH of bacon—first sampling with:		
Days from slaughter to cure	15	−0.01
Days in cure	20	−0.53*
Days from cure to first sampling	19	+0 05
pH of bacon—second sampling with:		
Days in cure	19	−0 39
Days from cure to second sampling	19	+0.53*
pH of bacon—first and second samplings with:		
Days from cure when measurement made for both samplings	40	−0.09
pH of bacon—first sampling with:		
Days in cure, independent of days from cure to first sampling	18	−0 51*
Days from cure to first sampling, independent of days in cure	18	0
pH of bacon—second sampling with:		
Days in cure, independent of days from cure to second sampling	18	−0.40
Days from cure to second sampling, independent of days in cure	18	+0 53*

The correlation coefficients between pH and time in cure were negative, and significant at the first, but not at the second sampling. The coefficients between pH and time from cure were positive and significant only for the second sampling. Similar results were obtained when the opposing effects of time in cure and time from cure, were rendered independent by partial correlation. This indicates that the pH of bacon decreases during cure but increases during maturation.

The increase in the pH of bacon during maturation was confirmed by the results of a supplementary experiment in which ground samples of bacon were stored at 1 to 2° C. for some time. The final pH values attained varied from 6.0 to 8.3. It was also observed that the colour and final pH of the bacon appeared to be related. All samples at about pH 6 0 were grey, those at pH 7.0 greyish-brown to brown, while those at pH 8.0 or higher had retained their red colour. A further investigation of the effect of pH on colour change has been projected.

Difference Between Bacon and Pickle from Different Plants

In a previous paper (8) dealing with the chloride, nitrate, and nitrite contents of bacon and pickle, it was found that the differences between plants were usually the major source of variation. The properties and constituents considered in the present paper did not show such marked variation between plants. With respect to pH, significant differences between plants could be demonstrated for cover pickle, but not for the pump or spent pickle, or for the bacon. The oxidation-reduction potential measurements showed significant differences between the bacon and spent pickles from different plants.

The different practices followed in the different establishments were reflected by highly significant differences in the moisture content of the bacon, and the protein content of the cover and spent pickles.

The generalizations summarized in the above paragraph are evident from the results presented in earlier tables. Since individual plants seldom differed greatly from the general means, and since the factors considered in this paper could only be controlled indirectly, more detailed discussion of this source of variation is considered unnecessary.

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CANADIAN WILTSHIRE BACON

IV. CORRELATION BETWEEN CONSTITUENTS AND PROPERTIES OF BACON[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

Simple correlation coefficients computed between several observed properties and constituents of factory-cured Wiltshire bacon showed the following to be associated: nitrate and chloride contents; nitrite and chloride contents; nitrite content and pH; and moisture and chloride contents. The first two of these associations probably arise from the pickle compositions and curing practices followed in the plants. The last two associations suggest a certain degree of dependence between the two properties, i.e., the nitrite content of the meat increases with the pH, and the moisture content decreases as the chloride content increases. When the moisture content of the bacon was expressed on a salt-free basis, the correlation between the moisture and chloride contents was not significant. This indicates that curing practices favouring a high salt content do not result in the removal of more moisture from the sides.

Introduction

The chloride, nitrate, nitrite, and moisture contents, pH values, and oxidation-reduction potential of factory-cured Wiltshire bacon and the pickles used for its manufacture, have been reported in earlier papers of this series (3, 4). These papers also reported the degree of correlation between similar constituents in the pickle and product. This paper deals with the correlations between the measured properties of the bacon.

Procedure

The methods employed for making these measurements have already been described (5, 8). The computation of correlation coefficients (7) between the observed quantities over 44 sides, representing 2 sides from each of 22 packing plants, served to determine whether statistically significant correlations existed between the measurements. It must be recognized, however, that a significant correlation between two quantities merely demonstrates that they are associated, and does not necessarily indicate that one is dependent on the other. Even where it seemed reasonably certain that one quantity was dependent on another, it was frequently difficult to determine which of the two was the causal agent. Thus a highly significant negative correlation between the pH and salt content of bacon could indicate either that the salt penetration was more rapid in the more acid sides, or that the pH decreases as the salt content increases.

Results

Simple Correlation Coefficients

The simple correlation coefficients between these quantities appear in Table I. These coefficients were computed from the results obtained for each sampling at which the measurements were made, and also between the mean values for each constituent over all samplings for each of the sides analysed. This procedure was followed because certain of the observed differences between samplings were attributable to the differences between positions (4, 5) from which successive samples were taken, while others may have represented a real change with time. In consequence it seemed desirable to compute the correlation coefficients from the results obtained at each sampling independently. On the other hand, the variations between different sides for certain properties were small. In such instances it was felt that the variance attributable to over-all sampling and experimental error for any two properties being studied might be sufficiently large to mask any existing correlation. Consequently it was desirable to use in addition the mean values for each measurement over all samplings in order to reduce the variance attributable to sampling and analytical error.

TABLE I

SIMPLE CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 42, for all except bracketted value = 30. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling			
	First	Second	Third	Mean of all
Nitrate in bacon with:				
Chloride in bacon	0.30*	−0.26	—	0.40*
Nitrite in bacon with:				
Chloride in bacon	0.45**	0.28	—	0.38*
Nitrate in bacon	−0.05	−0.23	−0.04	−0.07
Moisture in bacon	−0.04	−0.04	−0.06	−0.02
pH of bacon	0.56**	0.30*	—	0.47**
Eh potential of bacon (El. 1)	—	0.13†	—	0.16
Eh potential of bacon (El. 2)	[0.10]†	0.37*†	—	—
Moisture in bacon with:				
Chloride in bacon	−0.56**	−0.46**	—	−0.60**
Nitrate in bacon	−0.48**	−0.06	−0.07	−0.25
pH of bacon	0.18	0.17	—	0.13
Eh potential of bacon (El. 1)	—	0.05	—	—
pH of bacon with:				
Chloride in bacon	0.09	−0.23	—	−0.04
Nitrate in bacon	0.04	−0.05	—	—
Eh potential of bacon (El. 1)	—	—	—	0.26

† *Correlated with mean nitrite content over all samplings.*
* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

It is evident that values for a number of possible correlation coefficients do not appear in Table I. Most of these omissions result from the fact that several of the determinations were not made at the third sampling. Since the observed oxidation-reduction potentials of the bacon are subject to some uncertainty for reasons already given (3), only a few computations relating this quantity to other properties were made.

The majority of the correlation coefficients reported in Table I are not significant and do not require comment. Significant positive correlations were obtained between the chloride and nitrate contents of the bacon at the first sampling and between the means over all samplings. These coefficients doubtless reflect the association between the concentration of these two substances in the curing pickles or between the curing practices used in different plants, and are therefore of little consequence.

A significant positive correlation was obtained between the nitrite and chloride contents at the first sampling, and between the mean nitrite and chloride contents over all samplings. There was also a significant positive correlation between the nitrite content and the pH of the bacon at all samplings.

Significant negative correlations were obtained between the moisture and chloride contents at all samplings, and between the moisture and nitrate contents at the first sampling. The only significant correlation involving the oxidation-reduction potential was obtained with nitrite at the second sampling, the No. 2 electrode being used. Since these measurements were uncertain, and those with Electrode 2 probably less reliable than those with Electrode 1 (3), further comment is unnecessary.

Partial Correlation Coefficients

The relation between the nitrite content and the pH and chloride content, and that between the moisture content and the chloride and nitrate contents, were investigated further by computing partial correlation coefficients between these quantities. The values obtained appear in Table II. The correlation coefficients between the nitrite and chloride contents, independent of pH, were positive and highly significant at both the first and second samplings independently, and also between the mean values for each side over all samplings. There is therefore little doubt that the nitrite content of these sides increased with their chloride content. It seems probable, however, that these two constituents are not dependent on one another but are merely associated through the pickle compositions or curing practices followed in the different plants.

The coefficients representing the degree of correlation between the nitrite content and pH, independent of chloride content, also indicate a definite association between these quantities, the nitrite content increasing as the pH increases. Certain evidence indicates that the pH of pork (2, 6) and the nitrite content of the bacon (4) are properties of the individual side and to that extent are independent of the curing practices followed. Consideration of these facts sug-

TABLE II

PARTIAL CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 41. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling		
	First	Second	Mean
Nitrite in bacon with chloride in bacon independent of pH of bacon	0.48**	0.59**	0.45**
Nitrite in bacon with pH of bacon independent of chloride in bacon	0.59**	0.39**	0.53**
Moisture in bacon with chloride in bacon independent of nitrate in bacon	−0.50**	−0.49**	−0.56**
Moisture in bacon with nitrate in bacon independent of chloride in bacon	−0.39**	−0.21	−0.01

** *Indicates 1% level of significance.*

gests that there may be a dependence of one of the properties on the other rather than a mere association. If this is so it seems likely that the nitrite content is dependent on the pH rather than the reverse, since it is highly improbable that the small quantities of nitrite present would have any effect on the pH of the bacon. Such a dependence could be explained in several ways, namely: a more rapid penetration of the nitrite from the tank pickle as the pH of the meat increases; a more rapid production of nitrite from nitrate at higher pH levels; or a decreased rate of combination of nitrite with the muscle proteins and pigments at high pH levels. It has been shown that a high electrical resistance is associated with a high pH, and a slow penetration of chloride (2). If the absorption of nitrites is comparable with that of chlorides it seems unlikely that the first explanation is adequate for the observed differences in pH. Likewise it would appear that the rate of combination of nitrite with the muscle pigments would not be affected appreciably (1) by the variations in pH at the levels observed (3) in these sides. On the other hand the rate of reduction of nitrates by bacterial activity might reasonably proceed more rapidly as the pH increased within the observed range.

The results in Table II show that the correlations between moisture and chloride contents of the bacon, independent of nitrate content, were highly significant at each sampling independently, and for the mean values over all samplings. These show quite definitely that the moisture content decreases as the chloride content decreases. The correlation between the moisture and nitrate contents, independent of chloride content, was significant only for the results of the first sampling. It appears therefore that although the nitrate content may have a slight independent effect on the moisture content of the bacon, it is of secondary importance compared with that of chloride.

Relation between Moisture and Chloride Contents

The decrease in the moisture content with increase in chloride content of the whole bacon may merely reflect the effect of the additional dry matter present as sodium chloride. On the other hand it may be due to this effect plus the influence of certain curing practices, if conditions favouring the absorption of greater quantities of chloride also favour the loss of additional quantities of moisture from the sides. In order to eliminate the direct effect of the chloride on the dry matter content, the moisture contents, as previously reported (3), were computed on a sodium chloride-free basis for each side. When expressed as such, the mean moisture content was found to be 74.03% with a standard deviation of 0.70%, as compared with a mean of 71.38% and a standard deviation of 1.58% (3) calculated on the basis of the whole bacon. It is therefore evident that the direct effect of variations in the chloride content was a major source of variation in the moisture content of different sides.

The correlation between moisture, on a salt-free basis, and sodium chloride content was not significant ($r = 0.23$, for 40 degrees of freedom). The negative relation between the chloride and moisture contents of whole bacon, noted previously, was therefore due entirely to the additional dry matter contributed by the sodium chloride. These findings, together with the fact that the moisture content of bacon on a sodium chloride-free basis was essentially the same as that of fresh pork, indicate that the net result of pumping and curing is to increase the salt content, with little, if any, loss of moisture.

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CANADIAN WILTSHIRE BACON

V. QUANTITATIVE BACTERIOLOGICAL STUDIES ON CURING PICKLES[1]

BY N. E. GIBBONS[2]

Abstract

Five combinations of diluent, salt concentration of medium, and incubation temperature have been used to study the bacterial content of curing pickles used for making Wiltshire bacon. On representative pickles from 16 plants the highest mean count was on 10% salt agar (brine dilution) incubated at 20° C. The lowest count was on nutrient agar incubated at 37° C. Counts on media containing no salt, 4% salt, and 10% salt (water dilution) with incubation at 20° C. gave intermediate values. Counts on spent pickles were higher than on cover pickles. Pump pickles showed a surprisingly high number of organisms.

The analytical error attributable to diluting, plating, and counting was relatively small compared with the other sources of variance. Under certain conditions the error of sub-sampling a small jar of pickle exceeds the error between replicate plates. Sampling and sub-sampling errors were therefore the primary factors limiting the precision of the determinations.

Statistical analysis of the results showed that the differences in numbers observed by the different methods were highly significant for all pickles from 16 plants, and that the bacterial content of the pickles from the different plants differed significantly over all media. The number of bacteria observed by the different methods was usually correlated, i e., pickles from a plant showing a high count by one method usually showed a high count by the other methods and vice versa. Nevertheless, it was possible to demonstrate a significant differential response of the bacteria in cover and spent pickles to the different growth conditions used. This suggests qualitative differences in the flora of the pickles. Of the several growth conditions tested, nutrient agar at 20° C. and 10% salt agar with brine dilutions appear to be the most suitable for demonstrating differential responses attributable to qualitative differences between the flora of different pickles.

Introduction

An outline of the investigation on Wiltshire-cured bacon has been given in a previous paper (2). The present paper deals with the quantitative bacteriological findings on representative curing pickles obtained from the various plants. Several combinations of growth conditions were used in order to obtain, in addition to the strict quantitative variations in bacterial populations, an estimate of the validity of this procedure for detecting qualitative differences in the bacterial flora of different plants.

Samples of the three pickles used for curing were obtained from each plant. The pump pickle, injected into the sides prior to cure, is usually a freshly prepared brine which might reasonably be expected to contain few bacteria. The tank pickle, used to cover the sides during cure, was analysed at the beginning and end of cure, these samples being designated "cover" and "spent" pickles respectively. Details of the design of the experiment and methods of

sampling and of shipping the pickle have been described previously (2).
Most pickles were sampled within an hour or two of receipt, although occasion-
ally it was necessary to hold them as long as 12 hr.   Pump pickles from 19
plants, and cover and spent pickles from 16 plants, furnished the data on which
this paper is based.

Statistical methods were used to reduce and assist in the interpretation of
the data (6).   Since the bacterial numbers showed great variation, both
between plants and the various cultural methods employed, the logarithms
of the numbers were used both for convenience and valid interpretation on a
statistical basis (1).

Cultural Methods

Lochhead (5) has shown that the count obtained on nutrient agar with
incubation at 37° C., as commonly used in most packing plants for control
purposes, is lower than that on nutrient agar or salt agar incubated at 20° C.
Nutrient agar containing 10% salt, following dilution in brine of the same
concentration, gave the highest counts, these being about 80 times greater
than those obtained on nutrient agar incubated at 37° C.   Water dilutions
were found to render up to 90% of the organisms in pickle incapable of growth.

TABLE I

METHODS USED TO ESTIMATE THE NUMBER OF BACTERIA IN CURING PICKLES

Method	A	B	C	D	E
Temp. of incubation, °C.	37	20	20	20	20
Diluent	Dist. water	Dist. water	Dist. water	4% NaCl*	10% NaCl*
Medium	Beef-extract agar	Beef-extract agar	Beef-extract agar + 10% NaCl	Beef-extract agar + 4% NaCl	Beef-extract agar + 10% NaCl
Period of incubation, days	3	7	10	7	10

* % = Gm. added to 100 ml. of liquid.

These facts formed the basis for adopting the methods outlined in Table I.
Method A was selected since it is commonly used for control purposes in
packing plants.   As a working hypothesis it was assumed that Method B
would yield some estimate of the organisms introduced by the fresh sides;
Method C, the salt-tolerating organisms capable of withstanding considerable
change in osmotic pressure; Method D, the organisms capable of rapid growth
on cured bacon; and Method E, the halophilic flora of the pickle.   It is recog-
nized that these methods may overlap considerably and that they do not
yield an accurate estimate of the various types present.   They were, however,
considered suitable for a preliminary investigation of the subject.

Sampling

Since it was impossible to visit each of the plants, the samples were taken
by a plant operator, who was provided with detailed instructions.   By obtain-
ing two samples of the pickle (taken at different times) from each plant,

it was possible to obtain an estimate of the combined error due to sampling and to any differential change that occurred in the samples during shipment. A discussion of this phase of sampling has been given (2).

Apart from any systematic change that may occur in the bacterial numbers during shipment at different temperatures over varying periods of time, it is of interest to point out that a sub-sampling error arising from the removal of duplicate samples from a small jar of pickle was greater in some instances than the analytical error of plating and counting. The results of typical experiments appear in Table II. It can be seen that the sub-sampling error is significantly greater than the analytical error in two experiments at 22° C. and approaches significance in the third. Of the six experiments at 1.1° C., three of which were shaken more frequently during storage than the others, in only two was the sub-sampling error significantly greater. In both instances the variance necessary for significance was contributed by one set of duplicates. Since this error is apparently greater when pickle is stored at the higher temperature, it is possible that it is the result of protein precipitation which interferes with the uniform distribution of the organisms through the sample.

TABLE II

SAMPLING AND ANALYTICAL ERRORS OF COUNTS ON PICKLES STORED UNDER DIFFERENT CONDITIONS

Pickle	Method	Storage temperature, °C.	Sampling and analytical error		Analytical error		F
			D.f.	Mean sq.	D.f.	Mean sq.	
1	B	22	7	.0817	14	.0062	13.18**
		1.1	6	.0178	12	.0083	2.14
		1.1 (shaken)	6	.0148	12	.0099	1.49
2	B	22	7	.0249	13	.0094	2.65
		1.1	6	.0021	12	.0041	0.51
		1.1 (shaken)	6	.0047	12	.0012	3.77*
	E	22	7	.0378	28	.0044	8.59**
		1.1	6	.0086	24	.0027	3.18*
		1.1 (shaken)	6	.0091	24	.0177	0.51

* *Indicates 5%,* ** *1% level of significance.*

Quantitative Results

Typical arithmetic counts of cover pickles by the various methods are shown in Table III. The logarithms of the mean, maximum, and minimum number of bacteria per ml. over all plants are given in Table IV. In general the counts were maximal on 10% salt agar (Method E) and decreased with decreasing salt concentration (Methods D and B) and with increasing temperature (Method A). Dilution with water and incubation on 10% salt medium (Method C) gave results comparable with Method B. The mean

TABLE III

REPRESENTATIVE BACTERIAL COUNTS ON COVER PICKLES UNDER DIFFERENT CULTURAL
CONDITIONS. NUMBER OF ORGANISMS PER ML.

Sample	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	Dist. water 10% salt agar 20° C. (C)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)
1	700	5,000	7,400	60,000	900,000
2	75,000	96,000	57,000	300,000	470,000
3	12,000	36,000	19,000	61,000	120,000
4	46,000	150,000	170,000	900,000	4,700,000
5	70	600	500	770	600
6	630	12,000	11,000	30,000	34,000
7	39,000	130,000	63,000	500,000	490,000

TABLE IV

BACTERIAL COUNTS OF CURING PICKLES UNDER DIFFERENT CONDITIONS OF GROWTH

	Logarithm of number of organisms per ml.				
	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)	Dist. water 10% salt agar 20° C. (C)
Pump pickle					
Mean	3.01	3.69	4.25	4.40	3.78
Maximum	4.65	4.89	5.40	5.98	4.89
Minimum	1.68	2.40	2.43	2.15	2.13
Stand. dev.	.71	.65	.88	1.10	.78
Coeff. of variation	23.6	17.6	20.7	25.0	20.6
Cover pickle					
Mean	3.63	4.36	4.96	5.33	4.29
Maximum	5.00	5.36	6.24	7.12	5.48
Minimum	1.14	2.11	2.23	2.46	2.21
Stand. dev.	1.09	.87	1.07	1.23	.87
Coeff. of variation	30.0	20.0	21.6	23.1	20.3
Spent pickle					
Mean	4.44	4.99	5.68	5.95	4.88
Maximum	5.22	5.78	6.89	7.15	5.87
Minimum	3.26	3.85	4.35	4.47	3.68
Stand. dev.	.51	.44	.56	.66	.51
Coeff. of variation	11.5	8.8	9.9	11.1	10.4

number of bacteria in cover and spent pickles from all plants shows that
Methods A, B, and D measure about 2.5, 10, and 50%, respectively, of the
number observed by Method E. This agrees quite well with Lochhead's
figures of 1.3, 5.7, and 52.3% obtained from a number of observations on
the curing pickle of one plant (5).

On all media the mean count increases in the order, pump, cover, and spent
pickle. It is interesting to note that the freshly prepared pump pickle con-
tains, on the average, almost as many organisms as the cover pickle. In fact,
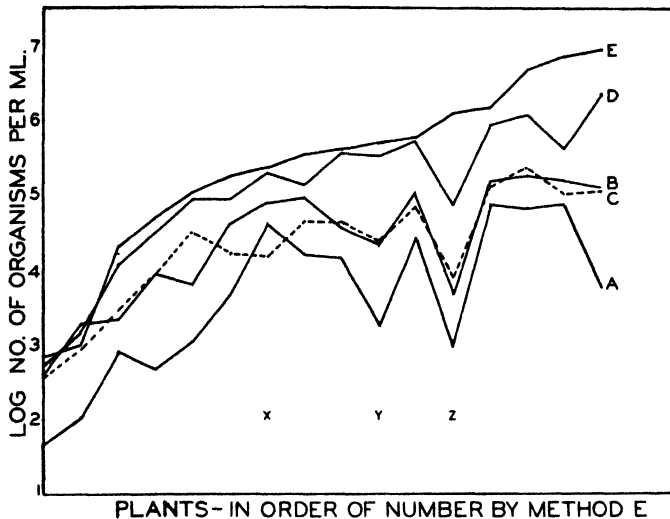
FIG. 1. *Bacterial content of cover pickle from 16 plants as indicated by different cultural methods: A—nutrient agar incubated at 37° C., B—nutrient agar incubated at 20° C.; C—10% salt agar from water dilutions; D—4% salt agar; and E—10% salt agar from brine dilutions. All incubated at 20° C.*

the minimum values for pump pickle are usually higher than those for cover pickle.

The maximum and minimum values, the standard deviation and the coefficient of variation (Table IV) give some indication of the variation that occurs between the various counts and pickles. Since the standard deviation usually increases with the mean number present by each method, the coefficient of variation is relatively constant for each type of pickle. The standard deviation and coefficient of variation indicate that counts on nutrient agar at 20° C. are the least variable, both absolutely and relatively, while the 10% salt agar count is relatively the most variable for pump pickle and the 37° C. count relatively most variable for cover and spent pickles. It is evident that the spent pickle is the least variable of the three types. It would seem that the number of bacteria present in cover pickle reflects the many variations in pickle formula and plant practice, but during cure becomes more uniform.

Sources of Variability

The variability indicated by the standard deviation in Table IV can be divided into that originating from three main sources: (i) combined error of dilution, plating, and counting (analytical error); (ii) combined error of sampling the tank, differential changes during shipment, and sub-sampling the jar of pickle (sampling error); and (iii) the difference in count of pickles from different plants. Since the number of organisms in the pickle may vary from time to time in the same plant, the sampling error is probably overestimated.

The analysis of variance of the counts on the three types of pickle is shown in Table V. In all cases the sampling error was significantly greater than

TABLE V

ANALYSIS OF VARIANCE OF BACTERIAL COUNTS ON PICKLES

Variance	Dist. water nut. agar 37° C. (A)		Dist. water nut. agar 20° C. (B)		4% brine 4% salt agar 20° C. (D)		10% brine 10% salt agar 20° C. (E)		Dist. water 10% salt agar 20° C. (C)	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump pickle										
Analytical error	71	.0042	72	.0014	74	.0020	72	.0023	74	.0020
Sampling error	19	1.1564**	19	.4593**	19	.3645**	19	.5663**	19	.5337**
Between plants	18	1.8093	18	2 0360**	18	4.3358**	18	6.7464**	18	3.1623**
Cover pickle										
Analytical error	64	.0142	61	.0022	62	.0012	61	.0026	61	.0034
Sampling error	16	1.1579**	16	.6007**	16	.2541**	16	.8702**	16	.8003**
Between plants	15	6.3017**	15	4.0321**	15	6.0120**	15	8.2778**	15	3.7652**
Spent pickle										
Analytical error	59	.0019	62	.0028	61	.0049	61	.0056	61	.0016
Sampling error	16	.3377**	16	.2597**	16	.2468**	16	.2741**	16	.2958**
Between plants	15	1.2070**	15	.9020**	15	1.6128**	15	2.3336**	15	1.2660**

** *Indicates 1% level of significance.*

the analytical error. Most of the variance attributable to sampling was contributed by a few plants showing considerable differences between the two samples. In spite of the large sampling error the difference between the bacterial numbers present in the pickles from different plants was highly significant in all but one instance. It can therefore be said with assurance that the bacterial numbers present in the pickles vary much more between plants than within plants, since the latter source of variance is included in the sampling error.

Relations of Counts by Various Methods

The numerical differences observed by the various methods may result from (i) essentially similar floras in all pickles with a fixed proportion of the maximum observed population developing under the different conditions, or (ii) different floras with a different fraction of the total population favoured by each method. Obviously a high degree of correlation between the quantitative counts by the several methods would indicate the first condition, while no correlation would suggest the second. Preliminary examination of the data indicated that there was some evidence favouring both of the above behaviours. For instance, a pickle having a high count by one method usually had high counts by all methods, suggesting a correlation between them. On the other hand, although the number of bacteria observed on nutrient agar incubated at 37° C. was on the average 2.5% of that observed on 10% salt agar incubated at 20° C., for the individual pickles this proportion varied from 0.08 to 16%. Although a general association exists between the bacterial numbers observed by the different methods for each cover pickle,.

TABLE VI

CORRELATION BETWEEN NUMBER OF BACTERIA GROWING ON VARIOUS MEDIA

Quantities correlated			Correlation coefficient (r)		
			Pump pickle[1]	Cover pickle[2]	Spent pickle[2]
Number on nut. agar 20° C.	*and*	Number on nut. agar 37° C.	.92	.94	.85
		4% salt agar	.89	.91	.90
		10% salt agar	.86	.86	.77
		10% salt agar (water)	.94‡	.93	.92
Number on 4% salt agar	*and*	Number on nut. agar 37° C.	.74†	.90	.81
		10% salt agar	.97‡	.91	.81
		10% salt agar (water)	.94‡	.96	.91
Number on 10% salt agar	*and*	Number on nut. agar 37° C.	.72†	.81	.70
		10% salt agar (water)	.94‡	.92	.83
Number on nut. agar 37° C.	*and*	Number on 10% salt agar (water)	.83	.87	.80

[1] *17 degrees of freedom, r of .58 required for 1% level of significance.*
[2] *14 degrees of freedom, r of .62 required for 1% level of significance.*
‡ *Significantly different from* †.

as shown in Fig. 1, there are some wide fluctuations as indicated when the numbers obtained from the plants marked *y* and *z* are compared with those from plant *x*.

In order to place these observations on a more quantitative basis, correlation coefficients were computed between the numbers of bacteria observed by the different methods. The values obtained appear in Table VI. All of these coefficients are statistically significant, thereby demonstrating a definite association between the numbers of bacteria indicated by the different methods. In other words, a pickle having relatively large bacterial numbers by one method will generally have relatively large numbers by all methods. On the other hand, the magnitude of the correlation coefficients is such that the residual variance unaccounted for by the coefficient varies from 5 to 50% of the total variance. This shows that there is a considerable element of independent fluctuation between the numbers observed by the different methods. These independent fluctuations responsible for the residual variance may be attributable wholly, or in part, to experimental error, or, as suggested earlier, to the possibility that the various cultural methods are capable of distinguishing to some extent a real difference between the floras present in different pickles. In order to determine the significance of these observations it was necessary to determine the differential response of the bacterial population to different cultural methods in a manner which permitted comparison with the known experimental errors. This suggested a variance and regression analysis of the data.

The differential response of the flora in the different pickles to the different cultural conditions is included in the interaction mean square, plants × methods (Table VII). This interaction should be tested for significance by comparing it with the variance between aliquots. Since duplicate determinations were not made on each medium this comparison was impossible. However, it has been shown (Table II) that for pickles kept at low temperatures the error between aliquots (sampling and analytical error) was seldom significantly greater than the analytical error alone, and in consequence the largest analytical error observed was used for comparison. On this basis the interaction mean square was found to be overwhelmingly significant.

TABLE VII

VARIANCE AND REGRESSION ANALYSES OF COUNTS BY ALL METHODS

Nut. agar 37° C.; nut. agar, 4% and 10% salt agar at 20° C.; 10% salt agar from distilled water at 20° C.

Variance	Pump		Cover		Spent	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Between plants	18	15.9807**	15	11.3190**	15	6.3109**
Between media	4	33.5442**	4	94.6674**	4	36.2393**
Differential plants × media	72	.6703**	60	.7307**	60	.3137**
Greatest analytical error	71	.0042	64	.0142	61	.0056
Greatest sampling error	19	1.1564	16	1.1579	16	.3377
Regression						
Differences in regressions	18	12.2352**	15	8 4022**	15	3.5119**
Residuals	57	1.2185	48	2.8537	48	1.2554

*** Indicates 1% level of significance.*

The fact that this plants × media interaction derived from an analysis of variance was statistically significant does not provide definite evidence that the organisms present in the different pickles respond differentially to the different growth conditions. The magnitude of the interaction may be affected by the number of organisms present in the pickle as well as by differential effects attributable to differences in flora. Since the number of organisms in the different pickles has been shown to differ significantly, it is necessary to determine the true differential effect from a regression analysis (7). It was then found (Table VII) that the differences between regressions accounted for the major portion of the interaction variance in pump pickle. There is, therefore, little evidence to indicate that different types of floras are present in this pickle. For cover and spent pickles the variance due to differences in regressions, although significant, accounts for only about half the interaction variance. The remainder is therefore attributed to differential response of the organisms to different growth conditions. This finding suggests some difference in the types of organisms present in the different pickles.

The various cultural methods included the effect of salt concentration, temperature of incubation and dilution with water and 10% brine. A further study was made to determine which of these factors contributed the most variance to the observed interaction (Table VII). The results, appearing in Table VIII, show that an increase in salt concentration from 0 to 10% in the medium contributes significantly more to the interaction than increasing the incubation temperature from 20° to 37° C. with a salt-free medium. The effect of dilution method (water or 10% brine) has a smaller differential effect than the salt concentration in the medium but a somewhat greater effect than the incubation temperature.

TABLE VIII

INTERACTIONS OF PAIRED METHODS SHOWING EFFECT OF TEMPERATURE OF INCUBATION, AND SALT CONTENT OF MEDIUM AND DILUENT

		Effect of					
		Temperature of incubation		Salt conc. of medium		Salt conc. of diluent	
		D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump	Interaction	18	.1722†	18	1 2458‡	18	.6294‡
	Greatest anal. error	71	.0042	72	.0023	72	.0023
Cover	Interaction	15	.4259†	15	1.2377‡	15	.9579
	Greatest anal. error	64	.0142	61	.0026	61	.0034
Spent	Interaction	15	.1650†	15	.5304‡	15	.9553‡
	Greatest anal. error	62	0028	61	0056	61	.0056

All interactions significant to 1% level.
‡ *Significantly different from †.*

These results show that the bacteriological difference between corresponding pickles from different plants is mainly quantitative. Cover and spent pickles, however, also show some differential response when cultured by different methods, suggesting qualitative differences in the flora. Media containing no salt and 10% salt incubated at 20° C. appear to be the most effective for demonstrating this differential response.

These findings are in agreement with practical conclusions. Since pump pickle is made in all plants from similar ingredients, quantitative rather than qualitative differences might be expected between different plants. However, because of the many methods of reclaiming spent pickle and the varying sources of contamination for cover pickle some differences in the types of organisms found in these pickles in different plants are possible.

Correlation of Bacterial Count with Chemical Composition of Pickles

Since bacterial activity is considered responsible for some changes in pickle composition during cure, correlation coefficients were computed between the differences in the logarithms of the number of bacteria present in the cover and spent pickles, or the relative growth rate during cure, and several constituents of the pickle (3). The correlations between the growth rate observed

in 4 and 10% salt media incubated at 20° C. and the mean value of certain components of the pickles appear in Table IX. None of these correlations was statistically significant for the 15 degrees of freedom available. Nevertheless the coefficient between pH and the relative growth rate was high enough to suggest some relation, the growth rate increasing as the pH increases within the range of observed values (4). Computation of this correlation on the basis of the results from individual pickles did not yield significant values, although twice as many degrees of freedom were available. Correlations between the arithmetic increase in bacterial numbers during cure and the nitrate and nitrite contents and the pH of the pickles were also insignificant.

TABLE IX

CORRELATION BETWEEN BACTERIAL GROWTH RATE AND MEAN VALUE OF CHEMICAL CONSTITUENTS OF COVER AND SPENT PICKLES

Quantities correlated			Correlation coefficient (r)
Log difference of counts on spent and cover pickles (4% salt agar)	*and*	Salt	$-.01$
		Nitrate	$-.12$
		Nitrite	$.02$
		pH	$.45$
		Protein	$-.33$
Log difference of counts on spent and cover pickles (10% salt agar)	*and*	Salt	$.19$
		Nitrate	$.22$
		Nitrite	$.11$
		pH	$.36$
		Protein	$-.01$

15 degrees of freedom, r of 0.48 required for 5% level of significance.

Actually a correlation with salt content could hardly be expected. The salt content of pickle varies from about 22 to 28%, and for halophilic organisms variations in salt content at concentrations about 20% have little effect. On the other hand, organisms affected by salt concentration are usually inhibited by concentrations much below this. A correlation between the increase in the number of bacteria, or of nitrate reducing organisms, and the total nitrate reduced might be expected, but under plant conditions it is very difficult to differentiate between the nitrate reduced and that absorbed by the sides since the combined errors applicable to the several determinations are large compared with the quantity of nitrate reduced. Likewise a measure of the total nitrite formed is difficult since the amount that reacts with the muscle proteins is unknown. Until these total quantities can be determined correlations of chemical constituents and bacterial numbers of pickle seem unlikely.

Acknowledgments

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CANADIAN WILTSHIRE BACON

VI. QUANTITATIVE BACTERIOLOGICAL STUDIES ON PRODUCT[1]

By N. E. Gibbons[2]

Abstract

Surface counts of bacteria on the ribs of bacon showed that nutrient agar containing 4% salt incubated at 20° C. gave the maximum number. In sampling the surface of a side of bacon, it was found that the removal of the surface layer of tissue yielded more accurate values than methods based on the removal of organisms by swabs or filter paper impressions.

On the average, the bacterial load on the anterior ribs was greater than on the posterior ribs. Although there were significant differences in the number of bacteria on sides from the same plant, the greatest variation was between sides from different plants.

A visible growth of bacteria or "slime" becomes evident on the average when the logarithm of the number of organisms per sq. cm. exceeds 7.2. Nevertheless, certain sides may appear slimy at log 6.7 per sq. cm., while others will not show this condition at log 8.0 per sq. cm. This variation in the number of organisms present at the visible slime level may result from differences in the flora, different types of growth of the same organism, or variability in the method of detection.

The number of bacteria on the side was found to be correlated with the age of the sides from cure or from packing. The growth rate is slow during the first 8 to 10 days from packing, after which it increases. Sides having an initial load of 100,000 organisms per sq. cm. at packing may be expected to remain free from slime for 20 to 25 days, if stored at 1.1° C. No correlation was obtained between the number of bacteria in the curing pickle and the number on the product.

Introduction

Wiltshire sides are matured for a week to ten days after removal from the curing tank. Whether bacteria play a part in this process is not known, but there is a development of organisms on the surface of the meat which eventually may lead to undesirable features, such as slime or taint.

This study was undertaken to determine the variation in the bacterial load on sides of factory-cured Canadian Wiltshire bacon at the time of export, and the effect of transport or storage on the increase in bacterial numbers and the appearance of slime. For this purpose two sides from each of 22 plants were examined. The treatment of these sides, both before and after reaching the laboratory, has been described elsewhere (2).

Cultural Methods

Some preliminary work was necessary to determine the best medium for growth and the best method of enumerating the bacteria on the surface of bacon. Many of the types found in pickle must be present on the sides when

removed from cure. While conditions may not be favourable for the development of all types present, some are able to grow as shown by the production of slime.

It was found that bacon infusion agars yielded slightly higher counts than beef extract agars, but the difficulty in preparing a clear medium, and the fact that the infusions contained varying amounts of salt, precluded their use as a routine medium. Beef extract, peptone agars were therefore used throughout the investigation.

To determine the optimum salt concentration, samples taken from cured bacon were diluted in brine containing from 0 to 10% salt, cultured on beef extract agar containing the same concentration of salt, and incubated at 20° C. The highest counts were obtained in the range 3 to 6% salt, the numbers falling off rather sharply at higher and lower concentrations. Within this range the maximum usually occurred on 4 to 5% salt. Since there is some advantage in using the lowest salt concentration compatible with good growth, a concentration of 4% salt was chosen with dilution in brine of the same concentration. It is of interest to note that the salt concentration of the bacon is usually about 4 to 5% (3). Consequently, it is evident that the conditions on bacon favour the development of organisms of intermediate salt tolerance.

Occasionally, counts equal to, or even higher than, those on 4% salt were obtained on 10% salt agar. It is conceivable that organisms from pickle, which prefer or are capable of growing at higher concentrations, sometimes persist for a time on the product.

Sampling

Three methods of taking the samples for surface bacterial counts were studied. The first of these, described by Haines (7), consists of outlining a definite area with sterile borers, and then removing a thin layer of the surface meat, or, in the case of ribs, the pleural membrane. Stainless steel borers, cutting a 3 sq. cm. circle, were used in this work. The second method, described by Garrard and Lochhead (4), consists of pressing a piece of filter paper of known area against the surface of the meat for 20 to 30 sec. The third was a swab method, with a piece of sheet aluminium having a 1 × 2 cm. opening used as a mask. In practice the mask was placed against the meat and the exposed area swabbed vigorously with two pledgets of absorbent cotton. The pieces of excised tissue, filter paper, or cotton obtained were in each instance transferred to dilution blanks containing coarse silica sand and shaken vigorously 100 times.

These methods were compared by taking duplicate samples by each method from the 3rd, 5th, 7th, 9th, and 11th ribs of a side of bacon. The position on the rib, from which each of the individual samples was taken, was determined by preparing a random sampling chart. The results were subjected to an analysis of variance which appears in Table I. The mean counts by the three methods show that the numbers obtained by the Haines and swab

techniques are in close agreement on the average, while that by the filter paper procedure is significantly lower. The mean square between duplicates shows that the precision decreases in the order: filter paper impression, Haines, and swab techniques. However, on the small number of observations made, only the swab technique can be said to be significantly less precise than the other methods.

TABLE I

ANALYSIS OF VARIANCE OF COUNTS ON BACON BY THREE METHODS

Method	Log mean no./cm.^{2}	Variance between duplicates		Variance between ribs		F
		D.f.	Mean sq.	D.f.	Mean sq.	
Haines	5.41	5	.028	4	.086	3.07
Filter paper	4.98	5	.013	4	.240	18.46**
Swab	5.40	5	.138	4	.041	0.30

** *Exceeds 1% level of significance.*

The filter paper impression method alone showed a significant difference between the numbers of bacteria on different ribs. Since the other two sampling procedures yielded similar results and showed no differences between ribs, it is concluded that the filter paper method, although precise, gives a less accurate estimate of the actual number of bacteria present. Examination of the detailed results showed that on an arithmetical basis the filter paper method gave about 75% of the count obtained by the Haines method on the 3rd rib and only 15% on the 11th rib. Since a similar gradient was not observed by the other methods on this particular side, and as the rear ribs are usually drier than those nearest the shoulder, it appears that the filter paper method may be affected by moisture conditions on the surface.

This effect of moisture was also observed for cut surfaces of meat. Pieces of filter paper of sufficient size were used to permit a sample to be taken afterwards from the same area by the Haines technique. On somewhat dry surfaces only about 10% of the organisms were removed by the filter paper when compared with the total number removed by the combined methods. On moister surfaces, up to 30% of the organisms could be removed by the filter paper.

The criticism may be made that on meat surfaces the Haines method may also include organisms from the sub-surface layers. However, similar results were obtained on rib surfaces where only the pleural membrane is removed. Since this membrane is not disintegrated when shaken with sand, only surface organisms would be removed.

The swab method is the least precise of the three. It should be mentioned that only after considerable experience with it could the high counts reported be attained. The Haines technique was therefore adopted in this study. Although it has the disadvantage of disfiguring the sides, it gives the best estimate of the actual number of organisms present, and in practice is probably the simplest to use.

Routine Procedure

In the examination of the sides from the various plants all counts were made on the pleural membrane, since this surface has the least opportunity of outside contamination, and slime generally appears there first. Areas of 6 sq. cm. were removed from the pleura over the 2nd and 3rd ribs and over the 9th and 10th ribs, and 3 sq. cm. from the 5th rib. The 5th and 6th ribs were then removed without contamination of the pleura and stored at 1.1° C. in an atmosphere of about 95% relative humidity (over saturated zinc sulphate solution) for 15 to 17 days. The sides were rebaled and stored at 1.1° C. for 10 to 12 days as previously described (2).

At the second sampling 6 sq. cm. samples were taken from all ribs. In all cases dilutions were made in 4% brine and cultured on 4% salt agar. Counts were made after 7 days' incubation at 20° C.

Quantitative Results

Statistical methods (8) have been used to interpret some of the data. Since there was a wide variation in the number of bacteria found, the numbers were converted to logarithms both for convenience and valid statistical analysis (1).

The logarithms of the mean, maximum, and minimum number of organisms per sq. cm. are shown in Table II. From the means it may be seen that there was a slight decrease in bacterial numbers on the pleura from the anterior to posterior ribs both before and after storage. At both samplings the counts for the anterior ribs showed the greatest variation. Since the 5th rib was stored for a longer period, the second sampling from it cannot be compared with the others.

TABLE II

BACTERIAL COUNT ON PLEURAL MEMBRANE OF WILTSHIRE SIDES

(Logarithm of number of organisms per sq. cm.)

	Ribs 2 and 3		Ribs 9 and 10		Rib 5	
	Received	Stored 10–12 days	Received	Stored 10–12 days	Received	Stored 15–17 days
Mean	4.54	6 03	4 21	5 18	4.51	6 94
Maximum	6.27	8 55	5.64	7.41	6 56	9.25
Minimum	2.97	3.48	2 90	3.19	3.42	4.21
Stand. deviation	.67	1.19	.55	.93	.60	1.16
Coeff. of variation	14.8	19.8	13.0	18.0	13.3	16.7

The maximum and minimum values, the standard deviation, and the coefficient of variation (Table II) give some indication of the variation that occurs between the different sides and also between ribs. Since the standard deviation decreases with the decrease in mean number present, the coefficient of variation is fairly constant.

Sources of Variability

The variability shown by the standard deviation in Table II may be divided into that arising from three main sources: (i) the error of diluting, plating, and counting; (ii) differences in the bacterial load of sides from the same plant; and (iii) differences in the product from different plants. The variance due to these three sources is shown in Table III. For all three positions on the side the variance due to differences between sides from different plants is significantly greater than that due to differences between sides from the same plant, which in turn is significantly greater than the experimental error. The greatest source of variability is therefore between plants.

Appearance of Slime

A side may be said to be slimy when the bacteria have increased sufficiently to be visible or tactile. Although not of serious practical importance unless very pronounced, slime is considered to be an indication of improper handling at some stage during the history of the product. If the numbers are sufficient to be discerned, it is probable that taint will soon be detected.

Many of the 5th and 6th ribs that had been stored at 95% relative humidity for 15 to 17 days showed slime, and furnished an opportunity of determining the number of organisms necessary for a visible growth. On arranging the values for slimy and non-slimy sides (Fig. 1), it was found that there was a region in which sides having the same count may or may not appear slimy. Pleural membranes having a count of 5,000,000 per sq. cm. (log 6.70) or less were never slimy; with a count of 93,000,000 per sq. cm. (log 7.97) or over, the pleura was always slimy. Between these limits there were nine slimy and nine non-slimy ribs having an average count of 16,500,000 organisms per sq. cm. (log 7.22 $\pm$ 0.093). It is therefore probable that any side having a count of 16 to 17 million organisms per sq. cm. will be slimy. The counts obtained on the sides stored for a shorter time confirmed these results (Fig. 2). Haines (6) has found that slime becomes visible on stored beef at a similar value (log 7.5).
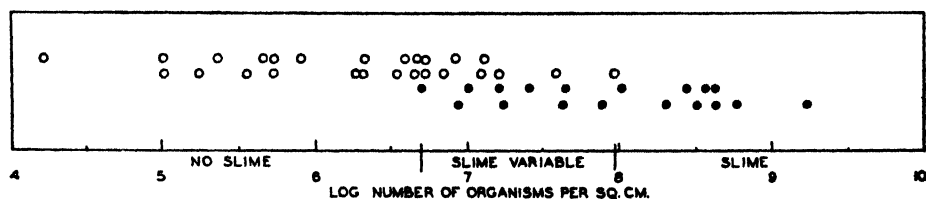
FIG. 1. *Relation between number of organisms on pleural membrane of Wiltshire sides and appearance of slime.*

In this study a visible growth was used as the criterion of slime. In practice a side may also be considered slimy if it feels slippery or if a growth can be detected by running the thumb nail over the surface. These subjective methods of detecting slime are doubtless liable to considerable variability,

TABLE III

ANALYSIS OF VARIANCE OF COUNTS ON BACON FROM 22 PLANTS

Variance	Ribs 2 and 3				Ribs 9 and 10				Rib 5			
	Received		Stored		Received		Stored		Received		Stored	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Analytical error	86	.0040	82	.0084	87	.0016	85	.0119	86	.0030	86	.0022
Between sides from same plant	22	.4671**	21	1.5617**	22	.2553**	22	1.0472**	22	.3641**	22	1.4774**
Between sides from different plants	21	2.2882**	20	7.0647**	21	1.5696**	21	4.1791**	21	1.8034**	21	6.6714**
Differential change with time between plants	20	2.507**			21	1.816**			21	2.339**		
Average change with time over all plants	1	127.545**			1	63.445**			1	397.096**		

**Exceeds mean square error or intra-plant variance, respectively, 1% level of significance.
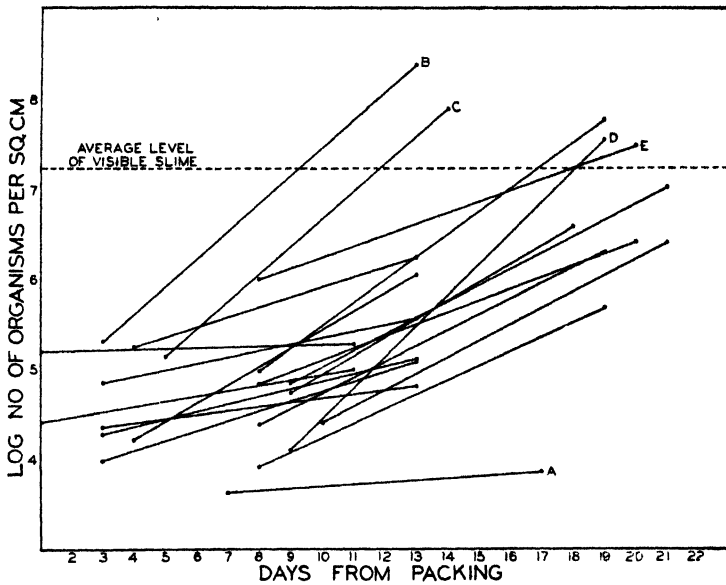
Fig. 2.   *Relation between number of organisms on pleural membrane of Wiltshire sides and age from packing.   (Sides B, C, D, and E showed definite slime.)*

even within a given method.   Consequently, variations in the number of organisms present when a side is considered to have become slimy are to be expected.   This may account for all or part of the observed variability. However, part of the variation may be real, since the visibility of equal numbers of organisms may be affected by the type of bacteria present, or by the conditions of growth on a particular side.

The significant interaction (differential change with time between plants, Table III) indicated that the bacteria do not increase at the same rate on sides from different plants.   This is apparent from the slopes of the lines in Fig. 2 and suggests that the flora of different plants varied.   It would appear that the flora of the sides from plant A is quite different from that of sides from plant B.   On the other hand, sides from plants B and C, and possibly D, seem to have a similar flora, which grows very rapidly and is soon apparent as slime.   Reports on the conditions of sides arriving in England showed that the product from plant B had a higher percentage of slime than that from the others.   The conclusion seems justified that, while the majority of plants apparently have very similar types of organisms present on the sides, in a few instances quite different types may be present.

Relation of Number of Bacteria Present to Time

A significant correlation coefficient indicated that there was a relation between the number of bacteria on the sides at successive samplings and the time from the end of cure to each sampling (Table IV).   The bacterial count was also correlated with the number of days from the beginning of cure and from packing.

On plotting the data (Fig. 2), this relation was confirmed and another demonstrated. Since the beginning and end of each line in Fig. 2 represent the number of bacteria present at the first and second samplings respectively, the slope of the line indicates the growth rate during this time. It can be seen that, if plants such as A, B, and C are excluded, the growth rate on sides sampled for the first time 2 to 4 days from packing is much less than on sides first sampled 8 to 10 days from packing. Although the lines are based on only two determinations, it is probably safe to conclude that under the conditions of storage there is a period of about 10 to 12 days during which the bacteria develop very slowly on the sides. After this time, development is much more rapid.

Since the appearance of slime depends on the number of bacteria present, it is evident that all sides will eventually become slimy. Apart from those having "abnormal" floras, the original load is important in determining how long the side remains free of slime. Sides having a high initial load (plant E) become slimy before those having a low initial load. From the evidence presented it may be said that, in general, if a side has a count of 100,000 per sq. cm. (about log 5) or less when packed, it will remain free of slime for 20 to 25 days under the usual conditions of transport.

TABLE IV

CORRELATION BETWEEN NUMBER OF BACTERIA ON PRODUCT, NUMBER IN PICKLE, AND TIME

Quantities correlated		Correlation coefficients (r)		
		Ribs 2 and 3	Rib 5	Ribs 9 and 10
Log of no. of organisms per sq. cm. on each side regardless of origin (total 40 sides) *and*	Days to each sampling from end of cure	.60**	.80**	.56**
	Days to each sampling from packing	58**	.80**	.57**
Log mean no. of organisms per sq. cm. on 2 sides originating from each of 20 plants *and*	Days to each sampling from end of cure	.64**		
	Days to each sampling from packing	.61**		
	Days start of cure to each sampling	.63**		
Log mean no. of organisms per sq. cm. on 2 sides from each of 20 plants (1st sampling) *and*	No. of bacteria per ml. in curing pickle at end of cure (4% salt agar)	.32†		
	(10% salt agar)	.25†		
	(Nut. agar 20° C.)	.28†		
	(Nut. agar 37° C.)	.14†		

** *Exceeds 1% level of significance, 38 degrees of freedom, r of .42 necessary for 1% level of significance for 35 D.f.*

† *15 degrees of freedom, r of .48 necessary for 5% level of significance.*

Relation of Number of Bacteria on Product to Number in Curing Pickle

The organisms found on bacon may come from a number of sources. Garrard and Lochhead (4) point out that the contamination on the sides prior to cure probably plays an important role. It is also possible that the organisms in curing pickle may play a part and the variation found in the number of bacteria in curing pickles (5) might be reflected in the number found on the product. To test this assumption the correlation coefficients shown in Table IV were computed. The number of bacteria on the bacon was not significantly correlated with the number of bacteria in the pickle in which it had been cured. However, the bacon examined varied in its age from cure and had been subjected to the various wiping and packing practices, and shipping conditions followed in the different plants. These factors, among others, are probably more important than the number of organisms in the pickle in determining the number of bacteria on the cured bacon.

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CANADIAN WILTSHIRE BACON

VII. SPECIFICATION OF COLOUR AND COLOUR STABILITY[1]

By C. A. Winkler[2] and J. W. Hopkins[3]

Abstract

A method of specifying the colour of bacon by measurement of its red, green, and blue spectral components is described.

Statistical evidence, based on a total of 792 observations, is presented which indicates that although the variation of these three components of colour from sample to sample of meat is to some extent correlated, each also exhibits a significant element of independent fluctuation. It is accordingly concluded that for investigational purposes, all three components should be included in forthcoming studies of colour and colour stability, and factors influencing them, in an extended series of samples. The possibility that for routine operations such as plant control a more limited analysis might suffice, will, however, also be made the subject of inquiry.

Introduction

Owing to the economic importance of the colour and colour stability of bacon exported from Canada, a study of these properties was included in a recent investigation (1) of some of the factors influencing Wiltshire bacon quality.

By visual inspection alone, it is possible to recognize considerable variation in the colour of different bacon samples, even when drawn from a single packing plant. In this case, the observed variation is probably due mainly to inherent differences in carcasses prior to curing. With product from different plants, the variability may be expected to be enhanced by differences in the curing practices employed. In these circumstances, it may be inferred that for an extensive study of the colour of bacon from various sources, a simple method of colour measurement, adaptable to routine procedure, is both adequate and desirable. A method by which only the red, green, and blue components of the colour are determined (5) was accordingly adopted.

Samples of meat may differ from one another in respect of either or both of two attributes of colour, namely chroma or colour quality, and total intensity or brightness. Variations in the former arise from differential reflection of one or more of the incident wave-bands by individual samples. Brightness,

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[3] *Statistician.*

on the other hand, is determined by the ability of the meat to reflect all components present in the incident light. Actually, the analysis of samples with respect to colour quality is believed to be the more important, as hue rather than intensity of colour seems to be primarily responsible for the observed variation in the initial appearance of different sides, as well as for the grey or brown discolorations sometimes developing on exposure to air.

In this investigation the percentage reflection of each colour component, relative to that of a white reference standard, provided information respecting colour quality. Total reflection was not measured specifically, but the sum of the percentage reflections for the three components yielded some indication of brightness. Certain complications must however be recognized in the relation of this index of brightness to visual appearance. Most artificial light sources, including that used in this study, are much richer in the red component than in the green or blue. As bacon is likewise predominantly red, much more of the red than of either the green or blue components is reflected from the meat surface. This combination of low incidence of green and blue light with the low reflecting power of bacon for these colours necessitated the use of much more intense illumination in the measurement of the percentage reflection of these than was required in the case of the red component. In fact, in order to obtain comparable degrees of precision, each of the three colour bands was studied at a different intensity of white light. This does not affect the estimation of the percentage reflection of each component, which is independent of the incident intensity, but does suggest that the summation of the three percentage values on equal terms may exaggerate the effect of differences in the blue and green components on the visual appearance of samples, even in white light.

Experimental

The colour measurements to be considered in this connection were made on samples from 44 Wiltshire sides, comprising two sides from each of 22 Canadian packing plants. Three samplings were made of each side, by methods described in the introductory paper of this series (1). On each such occasion two observations, namely of initial colour at the time of sampling, and of colour stability as indicated by the changes after exposure for 20 hr. at 10° C. and 95% relative humidity, were made on each sample, also by procedures previously outlined (5). There were thus 792 observations in all.

Chroma or Colour Quality

In earlier publications (5, 6), colour quality was defined in terms of intensity ratios, namely, red : green, red : blue, and green : blue. Statistical analysis of this property is, however, more simply accomplished by a consideration of the variance and covariance of the component intensities themselves, rather than of their ratios, comparisons of the latter being complicated by the fact that the ratios secured are not independent of the general intensity

level. This procedure will accordingly be adopted in the subsequent papers of this series.

It was not known a priori whether the measurement of all three of these components was actually necessary in order to specify the colour and colour stability of bacon. This would be the case if all three components were to vary to some extent at least independently from sample to sample of meat. On the other hand, if the fluctuations in the three components, although not necessarily of the same absolute magnitude, are nevertheless closely correlated, measurement of one component alone would suffice to specify all of them. Alternatively, an intermediate situation may be envisaged in which two of the colour components are closely associated, but the third varies semi-independently. As a first step in the examination of this question, the data accumulated were used to compute the coefficients of correlation between the red, green, and blue intensities shown in Table I.

TABLE I

COEFFICIENTS OF CORRELATION BETWEEN COMPONENT INTENSITIES OF COLOUR OF
INDIVIDUAL SAMPLES

Quantities correlated	First sampling	Second sampling	Third sampling	All samplings
Initial intensity				
Red × green	+.43	+.77	+ 81	+.68
Red × blue	+ 56	+ 42	+ 53	+.51
Green × blue	+.61	+ 82	+.87	+.80
Stability				
Red × green	+.05	+ 20	+ 92	+.85
Red × blue	−.04	+ 27	+.90	+ 80
Green × blue	+.57	+.72	+.94	+.91
5% point	± 30	± 30	±.30	±.16

Considering first the coefficients for initial intensity at each time of sampling, given in the upper portion of Table I, all 12 of these are statistically significant, indicating that there is some real association between the component intensities of any particular sample. It is to be observed, however, that the degree of association between both red and green, and red and blue, is in no instance very high, and when the measurements for all samplings are considered collectively, is indeed quite moderate. Determination of red intensity alone consequently would not provide a highly accurate index of either the green or blue observed in the same sample, and vice versa. The association between green and blue is, however, appreciably stronger, whether the three samplings are considered individually or collectively.

In considering the observations of colour stability, or more correctly, instability, which is the property actually measured, it requires to be noted that the changes in colour on exposure after smoking (third sampling) were

much greater than those observed on either of the other two occasions, and consequently dominate the results when these are considered collectively. For this reason, in spite of the insignificant correlation between red and green and red and blue at the first two samplings, the results as a whole indicate an association between the changes in intensity which is moderately high in the case of red and green and red and blue, and quite high in the case of green and blue.

The question next arises as to whether the residual variance not accounted for by the foregoing correlations may be regarded as arising solely from experimental errors, or whether there are genuine independent fluctuations in the individual intensities from sample to sample. In the absence of any direct, estimate of experimental error (single determinations only having been made on each sample) it is necessary to bring other evidence to bear on this point. This was done in two stages as follows:

The measurements of initial red, green, and blue intensity at all sampling times (396 observations) were first subjected to an analysis of variance (2) in which the main components of variation, arising from average differences between sides, between the intensity of the three colours, between the three sampling times, and the first-order interactions of these, were segregated from the second-order interactions of sides, sampling times, and individual colour intensities. As is pointed out by Fisher (3, sec. 41), such second-order interactions may be expected to be as a rule unimportant, and the variance apparently ascribable to them may accordingly be used to provide an estimate of experimental error, which will, however, be subject to inflation in proportion to the magnitude of any real interaction effects included in it.

When this was done, it was found that the residual variance of both green and blue, after correlation with red, was significantly greater than the foregoing interaction mean square, as judged by the usual variance ratio test (4). It would seem, therefore, that detectable variations in the intensity of blue and green, independent of red, may occur. The residual variance of blue after correlation with green did not exceed the interaction mean square, but owing to the possible inclusion of components other than error in the latter, this result is to be regarded as inconclusive, rather than definitely negative. In order to resolve this point, it is necessary to refer to computations, to be described in more detail in the next paper of this series, in which the homogeneity of the covariance of the green and blue intensity of individual samples within and between plants at each sampling time was examined in a manner described by Snedecor (4, sec. 12.3). By this means, significant variations in the blue intensity of samples from different plants, independent of concomitant variations in green, were demonstrable. It must be concluded, therefore, that there is in fact some element of independent variation in the intensity of each of the individual colours.

When examined in the same way, the measurements of change in colour on exposure yielded results paralleling the foregoing in that the residual variance of the change in both green and blue, after correlation with red, significantly exceeded the second-order interaction, and that the residual variance of change in blue after correlation with change in green did not.   In this case, however, the subsequent analysis of covariance did not demonstrate significant differences in blue change between the product of different plants, independent of change in green.

In view of these facts, it would seem that in the investigation of an extended series of samples, measurements should be made in all three spectral regions in order to specify colour quality, particularly if it is desired to relate this to chemical or other factors.   If only a limited number of samples is involved, the measurement of one component alone might suffice for comparative purposes at the present stage of development of the apparatus.   For routine purposes such as plant control, it likewise seems probable that the determination of a single component might prove to be adequate, although a decision in this connection must depend on the correlation of the results of instrumental analyses with those of visual inspection.   This point is currently receiving attention.

Colour Intensity or Brightness

As intimated above, the derivation of a satisfactory criterion of visual brightness from the instrumental readings made presents certain difficulties. As in the case of colour quality, it was not known beforehand whether the measurement of all three colour components was necessary, or whether one, or possibly two, of them would prove to be a satisfactory index of the total intensity.   It is true that, in earlier papers, the relative brightness of different samples was assumed to be proportional to the intensity of red scatter alone, but the results then available were inadequate to demonstrate the small but significant element of independent fluctuation of each of the individual components described in the preceding section.   On the other hand, in spite of this circumstance, certain considerations favour the use of the red intensity alone.   One of these, touched upon already, is the probable tendency of visual inspection to assess the brightness from the intensity of the predominant red colour.   Another is the fact that in addition to the independent differences noted above, there is also some degree of association between the intensities of the component colours of individual samples.

In the comparison of a limited number of samples, therefore, the red intensity may yield a satisfactory estimate of total brightness.   If large numbers of samples are to be studied, as in the present investigation, the sum of the three components would seem to be preferable.   A few measurements made with the apparatus without filters gave results about one-third the sum of the three percentage components obtained with filters, suggesting that the bright-

ness indicated by the latter is proportional to the intensity of reflection to be expected from a light source of the quality of a tungsten filament lamp.   It is, of course, arguable that a still better index of brightness would result from a summation in which the three percentages were weighted in proportion to the absolute intensities of red, green, and blue characteristic of the incident light.

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CANADIAN WILTSHIRE BACON

VIII. COLOUR OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

BY C. A. WINKLER[2], J. W. HOPKINS[3], AND M. W. THISTLE[4]

Abstract

Photoelectric measurements on two factory-cured Wiltshire sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between individual sides in respect of both total intensity and quality of colour, which would seem to have arisen mainly from differences between plants. The average range of variation between plants was: total intensity, 25%; red intensity, 23%; green, 30%; and blue, 35% of the mean. Differences in colour quality of two types, (i) due to variations in the component intensities which were correlated but not of the same absolute magnitude, and (ii) due to uncorrelated variation in the component intensities, were demonstrable.

Partial correlation studies led to the deduction of a moderate degree of association between colour quality, and pH and nitrite content, under the conditions of sampling (ii). Increased acidity was accompanied by an enhanced green and a depressed blue intensity. Increased nitrite content also tended to depress blue intensity, but apparently without significantly affecting the green. No correlation between colour and the salt, nitrate or moisture content of the meat was demonstrable.

Introduction

In this paper, some of the colour measurements made during the course of a survey of factors influencing the quality of Canadian Wiltshire bacon (1) will be discussed. As intimated in the preceding paper of this series (7), determinations were made on samples from two sides from each of 22 Canadian packing plants, or 44 sides in all. Each side was sampled, by the procedure described elsewhere (1) on three occasions, namely, (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C. The resulting 132 samples were examined in a photoelectric colour comparator (6), by means of which separate measurements were made of the intensity of the light in the blue (4,000-4,500Å), green (4,900-5,800Å), and red (5,750-7,000Å) spectral regions reflected, or more correctly, scattered, at right angles to the surface of the meat. The results secured thus fall under the two heads of total intensity or brightness, and chroma or colour quality, of which the latter is considered to be the more important for reasons already put forward (7).

In addition to the determination of colour at the actual time of sampling, measurements were also made of colour stability, as indicated by the change in colour of each sample after exposure for 20 hr. at 10° C. and 95% relative humidity. These will form the subject of a separate communication.

Colour Intensity or Brightness

It has already been shown (7) that there is some element of independent variation in the amounts of red, green, and blue scatter from sample to sample, and that measurements of all three should consequently be used in order to specify both total intensity and quality of colour. The figures for total intensity dealt with in this section are accordingly the sums of these three components in each case, although even these, for reasons indicated elsewhere (7), are probably not entirely satisfactory indices of visual brightness.

Analysis of Variance

The variance of the 132 observations of intensity or total brightness, as defined above, was analysed, by the procedure due to Fisher (5), into three portions, ascribable (i) to average differences, over all plants, between the three sampling occasions, (ii) to average differences, over the three samplings, between sides, and (iii) to the differential variation, or interaction, of sides with sampling time. The variance between sides was further allocated within and between plants, following which a corresponding partition of the interaction variance was also made. The results of this computation are shown in Table I, while Table II summarizes some of the main features of the actual observations.

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR BRIGHTNESS

Variance due to	Degrees of freedom	Mean square
Sides	43	76.3*
Between plants	21	97.2
Within plants	22	56.3
Sampling times	2	647.1**
Interaction sides × samplings	86	47.1
Between plants	42	55.0
Within plants (residual)	44	39.6

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

The analysis of variance indicates a definitely significant difference between sampling times in the average measurements for all 44 sides, due, however, almost entirely to the increased reflectivity of the samples taken after smoking (sampling (iii)). Moderately significant differences in the average bright-

TABLE II

SUMMARY OF MEASUREMENTS OF COLOUR INTENSITY OR BRIGHTNESS

Quantity	Sampling time		
	(i)	(ii)	(iii)
Maximum			
Individual side	104.5	102.5	110.7
Plant (av. 2 sides)	96.2	97.4	108.6
Minimum			
Individual side	74.8	74.2	72.9
Plant (av. 2 sides)	79.4	75.4	78.2
Mean	87.8	88.1	94.6

ness over all three samplings of individual sides are also demonstrable. These would seem to have arisen mainly from differences between plants, as although the number of observations available is not sufficient to establish the statistical reality of the difference in the inter- and intra-plant variance, the variance between sides drawn from the same plant does not significantly exceed the residual. This is a point of some interest, since the variability of sides from the same plant probably arises mainly from inherent differences in carcasses prior to curing, whereas between plants, differences in curing practice may be operative. On the other hand, there seems to have been no pronounced differential effect of smoking on the total colour intensity of the product from different plants, the interaction of plants × samplings (Table I), although suggestive, failing significantly to exceed the residual variance.

The means for the three samplings, shown in Table II, all fall approximately midway between the respective maximum and minimum plant averages, indicating that the values for the 22 individual plants were reasonably symmetrically distributed over the observed range of variation, without any marked bias in either direction. The average range between the maximum and minimum plant averages in the three samplings was 25% of the mean. Although doubtless susceptible of some improvement, this may perhaps be regarded as not unreasonable in view of the fact that the averages were, after all, deduced from only two sides from each plant, and further that the product of different plants was of varying age when received at the laboratory.

Correlation with Chemical Analyses

In order to ascertain whether there was any readily demonstrable association between total intensity of colour and chemical composition as determined by Cook *et al.* (2, 3), a number of simple correlation coefficients were calculated. With one exception, these proved to be uniformly insignificant. The results for sampling (iii), i.e., after smoking, yielded a coefficient of $r = -0.38$ between colour intensity and nitrite concentration in p.p.m. (5% point, $r = \pm 0.30$), but the coefficients for both the earlier samplings were insig-

nificant.   Nitrate and moisture content also gave insignificant coefficients for all three samplings.   Salt concentration and pH were examined only for the first two samplings.   Neither was correlated with intensity.   The partial correlation between intensity and pH, after allowing for associated variations in nitrite concentration, was likewise insignificant for both samplings (i) and (ii).

Chroma or Colour Quality

Analysis of Variance and Covariance of Component Intensities

Table III gives the results of an analysis of variance of the component red, green, and blue intensities of the individual samples paralleling that for total brightness.   The previously noted difference in total intensity between sampling times is seen to be the result of variation in all three components. Table IV shows, however, that the increase after smoking was greatest in the blue region of the spectrum and least in the red.   The analysis of variance likewise indicates that the observed differences between sides in respect of red scatter were no greater than would be expected from the residual variance in this respect of successive samples from the same side, which latter, however, is sensibly greater than the corresponding residual variance of either green or blue scatter.   On the other hand, the differences between sides in both green and blue are quite significant, and the inter-plant variance of blue demonstrably exceeds the intra-plant.   It is also to be noted that the increase in intensity of green and blue after smoking was not of the same magnitude in the product from all plants, the interaction of plants × samplings (Table III) being significant in both instances.   A complicating factor must be noted here, however, in that the sides from the eastern and western plants were smoked on different occasions, and apparently under somewhat different conditions.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	10.6	8.3**	13.8**
Between plants	21	11.3	10.6	24.0
Within plants	22	9.7	6.0	4.1
Sampling times	2	34.9*	68.7**	122.4**
	86	10.2	5.3	5.1**
Interaction sides × samplings				
Between plants	42	9.7	6.9*	8.3**
Within plants (residual)	44	10.6	3.8	1.9

  * *Exceeds mean square residual, 5% level of significance.*
 ** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum									
Individual side	48 6	32 7	29 2	46 6	30 1	30 0	44 4	34 0	33.3
Plant (av. of 2 sides)	41 2	31.2	26 2	43 8	29 0	29 0	43 2	32.9	32.6
Minimum									
Individual side	30.4	22 1	20 0	30 2	22 0	19 9	32 9	21 9	20.0
Plant (av. of 2 sides)	33 0	23.4	20.6	33.2	22 2	19 9	34 0	23 3	20.9
Mean	38 0	26 6	23 2	37.8	26 1	24 1	39.4	28 5	26.4

The means of each component intensity again fall about mid-way between the respective maximum and minimum plant averages in all three samplings. The range between plants, averaged over the three samplings, is 23% in red, 30% in green, and 35% in blue.

Reference has already been made (7) to the covariance of the component intensities from sample to sample of meat. In view of this, two types of variation in colour quality are possible: (i) within a homogeneous system, due to the fact that the increments in the component intensities associated with a given increase in total brightness, although correlated, are not of the same magnitude; and (ii) of an irregular nature, due to uncorrelated variations in the component intensities.

It was shown (7) that variations in colour quality of type (ii) were demonstrable, and in this connection an analysis of covariance was employed to establish the fact that there were significant differences in the blue intensity of the product from different plants, independent of associated variations in green, on all three sampling occasions. A further analysis of the covariance of the red and blue intensities of the six samples (from two sides at each of three sampling times) of product from each plant into portions within and between samplings (3 and 2 d.f. for each of 22 plants respectively) was also made. This indicated that there were significant differences in blue intensity, independent of red, between samplings.

These analyses also provided information respecting the effect on colour quality of the correlated, as well as the uncorrelated, variations in component intensities. Thus, the regression coefficients within plants calculated in the course of the first analysis indicated an average change of 0.67 units in blue intensity associated with unit change in green, and those within samplings determined in the second analysis, an average change of 0.31 units of blue per unit of red. It must be concluded, therefore, that variations in colour quality of both the types mentioned above occur in practice, and that those of type (ii) may be encountered either in the product of different plants, or in that of the same plant sampled before and after smoking.

Correlation of Component Intensities with Chemical Analyses

As a first step in the investigation of the relation between the component intensities of colour and the chemical properties of the meat, the simple correlation coefficients listed in Table V were determined. These indicate that above-average nitrite content was associated with below-average intensities of both green and blue. Otherwise, they are quite insignificant, with the exception of those for blue intensity and pH (sampling (ii)), and blue intensity and moisture content (all data).

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour measurement	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
sampling (i)	−.03	−.18	−.00	−.06	−.06
sampling (ii)	−.06	−.13	+.06	+.01	−.03
sampling (iii)	—	+.08	−.21	—	+.18
All data	+.05	−.05	−.05	−.02	−.12
Green intensity					
sampling (i)	−.14	−.22	−.32*	−.41	−.12
sampling (ii)	−.13	− 19	−.09	+.17	+.02
sampling (iii)	—	−.06	−.38*	—	+.16
All data	−.18	−.11	−.28**	−.09	−.20
Blue intensity					
sampling (i)	+.01	−.02	−.14	−.26	−.25
sampling (ii)	−.15	−.21	−.20	+.40**	−.02
sampling (iii)	—	−.13	−.41**	—	+.21
All data	+.05	−.07	−.29**	+.05	−.31**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

It has to be recognized, however, that simple correlation coefficients may fail to portray adequately the relation between colour quality and chemical composition. In the first place, the former is dependent in part on the general level of intensity, owing to the differential magnitude of the correlated variations in the three spectral components. Secondly, as has been pointed out by Cook and White (4), fluctuations from sample to sample in the chemical factors themselves are not all mutually independent. Both of these circumstances may operate either to obscure or to exaggerate the real relation between individual colour components and chemical qualities. For this reason, a number of third order partial correlation coefficients have been calculated, in order to examine the relation between pairs of factors independent of associated variation in certain others. These, which are confined to the results for samplings (i) and (ii), i.e., before smoking, will be found in Table VI.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated		Independent of		Correlation coefficient	
				Sampling (i)	Sampling (ii)
Intensity	*Analysis*	*Analysis*	*Intensity*		
Red	× salt	Nitrate,	blue and green	+.03	+ 03
		Nitrite,	blue and green	−.09	+.00
		Moisture,	blue and green	+.04	−.04
		pH,	blue and green	—	+.05
Red	× nitrate	Salt,	blue and green	−.18	−.01
		Nitrite,	blue and green	—	+.01
		Moisture,	blue and green	−.16	—
Red	× nitrite	Salt,	blue and green	+ 17	+.10
		Nitrate,	blue and green	—	+.10
		pH,	blue and green	+.07	+.06
Red	× pH	Salt,	blue and green	—	+.12
		Nitrite,		+.10	+.08
Red	× moisture	Salt,	blue and green	+.10	−.14
		Nitrate,	blue and green	−.00	—
Green	× salt	Nitrate,	red and blue	−.12	−.04
		Nitrite,	red and blue	−.04	−.03
		Moisture,	red and blue	−.21	−.02
		pH,	red and blue	—	−.09
Green	× nitrate	Salt,	red and blue	−.19	−.02
		Nitrite,	red and blue	—	−.02
		Moisture,	red and blue	−.26	—
Green	× nitrite	Salt,	red and blue	−.27	−.00
		Nitrate,	red and blue	—	−.01
		pH,	red and blue	− 17	+.18
Green	× pH	Salt,	red and blue	—	−.28
		Nitrite,	red and blue	−.21	−.30
Green	× moisture	Salt,	red and blue	−.10	+ 15
		Nitrate,	red and blue	−.11	—
					−.07
Blue	× salt	Nitrate,	red and green	+.07	
		Nitrite,	red and green	+.13	−.01
		Moisture,	red and green	−.02	−.12
		pH,	red and green	—	+.05
Blue	× nitrate	Salt,	red and green	+.18	−.10
		Nitrite,	red and green	—	−.11
		Moisture,	red and green	+.10	—
Blue	× nitrite	Salt,	red and green	−.04	−.12
		Nitrate,	red and green	—	−.15
		pH,	red and green	+.07	−.35*
Blue	× pH	Salt,	red and green	—	+.45**
		Nitrite,	red and green	−.11	+.53**
Blue	× moisture	Salt,	red and green	−.21	−.17
		Nitrate	red and green	−.15	—

* *Exceeds 5% point (r = 0.31).*
** *Exceeds 1% point (r = 0.40).*

They are again, for the most part, of quite negligible magnitude. Demonstrable effects are confined to the blue region of the spectrum, in which the intensity seems to be correlated negatively with nitrite content and positively with pH, i.e., the blue component increased with increasing alkalinity. There is also some indication of a negative association between green intensity and pH, but this falls short of significance. It is noteworthy that the main colour component, red, yields no indication of association with any of the chemical quantities determined.

In view of the indications obtained, the correlation of blue and green intensities with nitrite content and pH was further investigated by the calculation of still higher order partial coefficients from the results of sampling (ii). When this was done, the fourth order coefficient for green intensity and pH, independent of red intensity, blue intensity, salt content, and nitrite content, was found to be $r = -0.33$, which just exceeds the 5% point of $r = \pm 0.31$. Similarly, the fourth order partial correlation of blue intensity and pH, independent of red intensity, green intensity, salt content, and nitrite content, gave $r = 0.56$, and the fifth order partial correlation between blue intensity and nitrite content, independent of red intensity, green intensity, salt content, nitrate content, and pH, $r = -0.43$, both of which exceed the 1% point. There would thus seem to have been, under the conditions of sampling (ii) at any rate, a moderate degree of association between these two factors and colour quality, such that increasing acidity tended to raise the green and depress the blue intensity, and increasing nitrite content also tended to depress the blue intensity, but without affecting the green.

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CANADIAN WILTSHIRE BACON

IX. COLOUR STABILITY OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES[1]

By C. A. WINKLER[2], J. W. HOPKINS[3], AND E. A. ROOKE[4]

Abstract

Photoelectric measurements on samples of two factory-cured sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between the product of individual plants in respect of the stability of total intensity of colour, and of the component intensities of red, green, and blue, on exposure of freshly-cut samples for 20 hr. at 10° C., and 95% relative humidity. Apart from a batch effect after smoking, however, there was no marked segregation of any one plant or group of plants from the remainder in respect of colour stability of product. On the average, the effect of smoking was to reduce colour stability.

Analyses of covariance demonstrated (i) a significant degree of correlation between the green and blue stability of the same sample, and (ii) a further correlation between the initial green and blue intensity and the stability of these components, samples of higher initial intensity suffering a greater reduction on exposure. Partial correlation studies suggest that increased nitrite content was accompanied by an enhanced stability of the red component of colour, but no correlation between the salt, nitrate and moisture content or pH of the meat and its colour stability was demonstrable.

In the preceding paper of this series (7), some of the colour measurements made in a survey of factors influencing the quality of Canadian Wiltshire bacon (1) were discussed. It was explained that observations were made on samples from 44 sides, two from each of 22 Canadian packing plants, each side being sampled (i) upon receipt at the laboratory; (ii) after storage for 10 days at 1° C.; and (iii) after smoking for 14 hr. at 40° C. The measurements of colour stability, now to be discussed, were secured by determining the change in intensity and quality of colour of the 132 individual samples after exposure for 20 hr. at 10° C. and 95% relative humidity. Apparatus and procedure were the same as those already described (6, 7), and the results will be dealt with under the heads previously adopted in considering the measurements of initial colour.

Change in Colour Intensity or Brightness

For reasons advanced elsewhere (6), total intensity or brightness is defined as the sum of the separate intensities of the red, green, and blue spectral components, relative to the white standard.

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Analysis of Variance

The 132 individual measurements of change in total intensity or brightness as defined above, were subjected to an analysis of variance (5), of the same form as that applied to the observations of initial colour. The results of this are given in Table I, while Table II summarizes salient features of the measurements themselves. It will be observed that the mean values in Table II are all negative in sign, indicating an average decrease in intensity of colour of the samples on exposure.

TABLE I

ANALYSIS OF VARIANCE OF CHANGE IN TOTAL INTENSITY OF COLOUR

Variance due to	Degrees of freedom	Mean square
Sides	43	39.8**
Between plants	21	71.7**
Within plants	22	9.4
Samplings	2	3064.5**
Interaction sides × samplings	86	68.9**
Between plants	42	128.3**
Within plants (residual)	44	12.2

** *Exceeds mean square residual, 1% level of significance.*

From the analysis of variance, it is to be deduced that there were significant differences in the colour stability of the product of different plants, but that the variance between sides from the same plant did not exceed the residual. On the average, stability changed significantly between samplings, but the interaction mean square indicates that the product of individual plants behaved differentially in this respect. In reality, the variance between samplings is very largely due to the decrease in average colour stability after smoking

TABLE II

SUMMARY OF MEASUREMENTS OF CHANGE IN TOTAL INTENSITY OF COLOUR

Quantity	Sampling		
	(i)	(ii)	(iii)
Maximum change			
Individual side	−12.2	−11.3	−35.7
Plant (av. 2 sides)	−10.2	− 8.0	−34.4
Minimum change			
Individual side	+ 0.1	+ 0.1	− 0.8
Plant (av. 2 sides)	− 0.1	+ 0.1	− 1.4
Mean change	− 4.7	− 1.7	−17.4

(Table II), and the differential effect is likewise largely ascribable to the fact that this decrease was confined to the sides from the eastern plants, in which it was very pronounced, and absent from those from the western plants, which were smoked in two separate batches. Unfortunately, it cannot be stated whether this discrepant behaviour was due to differences in age or other inherent properties of the meat at the time of smoking, or to variation in the conditions under which the two batches were smoked. Examination of the individual observations in samplings (i) and (ii), however, does not suggest that any one plant or group of plants stood out from the remainder in respect of colour stability of product before smoking. Rather, there was a continuous series of plant averages from the maximum to the minimum observed values.

Correlation of Change with Initial Intensity

The correlation of the observed change in total intensity of colour on exposure with the initial intensity of the freshly cut samples was also investigated, for each sampling separately. The 44 individual samples in sampling (i) gave $r = -0.28$, and those in sampling (ii) $r = -0.18$, both of which fall short of the 5% point ($r = \pm 0.30$). On the other hand, the results for sampling (iii) as a whole gave $r = +0.40$, exceeding the 1% point of $r = \pm 0.38$. This, however, was again due to the fact that the sides from the eastern plants, smoked in the first batch, exhibited both a lower average initial intensity of colour, and also an appreciably greater average decrease in intensity on exposure. So much was this the case, that when the covariance was computed about the two batch means separately, rather than about the mean of all 44 samples, a significantly negative value of $r = -0.33$ was obtained, indicating that within batches, those samples having the highest initial intensity actually tended, on the average, to show the greatest decrease on exposure.

Correlation with Chemical Analyses

Simple correlation coefficients were calculated between the change in total intensity and the salt content, nitrate content, nitrite content, moisture content, and pH of individual sides, as determined by Cook, White, and Chadderton (2, 3), but in view of the differential behaviour of the eastern and western batches after smoking, these computations were confined to the results for the first two samplings.

The resulting coefficients were all insignificant with the exception of the one for nitrite content in sampling (ii). This gave $r = +0.37$, which exceeds the 5% point of $r = 0.30$ and thus suggests that increased nitrite content may be associated with stability of colour. The corresponding value for sampling (i), $r = +0.23$, failed to attain significance, but that for sampling (ii), at $+0.39$, was practically unaffected when recomputed as a first-order partial, eliminating the effect of accompanying variations in pH.

Changes in Chroma or Colour Quality

Analysis of Variance and Covariance of Change in Component Intensities

Tables III and IV show respectively the analysis of variance and range of fluctuation of the changes on exposure, in the individual intensities of red, green, and blue. All reproduce the main features already noted in the observations of total intensity, although the differences between plants in respect of blue stability are less pronounced than those in the red and green regions. Here also the interpretation of the differential behaviour of the sides from the eastern and western plants after smoking is obscured by batch effects.

The covariance of the changes in red, green, and blue intensity in individual samples has already been discussed to some extent in an earlier paper, which contained a tabulation of the correlation coefficients bearing upon this point (6, Table I). The results for samplings (i) and (ii) agreed in indicating

TABLE III

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	7.6**	7.0**	3.5
Between plants	21	11.2**	12.9**	5.3*
Within plants	22	4.1	1.4	1.8
Samplings	2	505.2**	390.2**	175.4**
Interaction sides × samplings	86	13.2**	9.8**	5.3*
Between plants	42	23.6**	17.5**	7.9**
Within plants (residual)	44	3.3	2.5	2.9

* *Exceeds mean square residual, 5% level of significance.*
** *Exceeds mean square residual, 1% level of significance.*

TABLE IV

SUMMARY OF MEASUREMENTS OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum decrease									
Individual side	−10 7	−3.8	−7.0	−6.7	−2.9	−3.5	−17.3	−11.7	−8.5
Plant (av. 2 sides)	− 7 6	−2.4	−4.0	−5.4	−1.4	−2.8	−15.9	−10.8	−8.2
Minimum decrease									
Individual side	− 0 4	+4.1	+2.6	+1.7	+2.5	+2.3	− 1.0	+ 1.7	+0.5
Plant (av. 2 sides)	− 0.8	+3.1	+1.2	+0.1	+2.0	+1.7	− 1.4	+ 0.9	+0.1
Mean change	− 3 8	−0.1	−0.8	−2.1	+0.5	−0.1	− 8.6	− 5.0	−3.8

a significant positive correlation between the changes in green and blue, but did not demonstrate any definite association of either of these quantities with red stability. Changes in all three intensities were significantly correlated in sampling (iii), but this must again be considered as a reflection of the batch effect mentioned above. It was also pointed out that there were significant variations in the stability of blue and green, independent of that of red, in the observations as a whole. This was, however, found to be no more pronounced in the sides from different plants than in those from the same plant, with the exception of green stability in sampling (i), which showed some variation from plant to plant independent of red.

A further analysis of the covariance of the red and green, and red and blue stability of the six samples (two sides at each of three sampling times) taken from the product of each plant, into portions within and between samplings, was also made. This paralleled a similar analysis of the actual intensities at the time of sampling, reported in the preceding paper (5). The results demonstrated (i) no significant element of correlation between the stability of the three colour components of individual samples of the bacon from the same plant, within samplings, but (ii) significant differences between samplings in the stability of both blue and green, independent of red stability. This effect was, however, confined to the samples from the eastern plants, and must accordingly be regarded as a further consequence of batch differences.

Correlation with Components of Initial Intensity

As in the case of total intensity, the correlation between the stability of red, green, and blue, and the initial intensity of these components of colour in the fresh samples was determined for each sampling.

The observations of initial red and change in red intensity in the two samplings prior to smoking gave $r = -0.21$ for sampling (i) and $r = +0.05$ for sampling (ii), both of which are statistically insignificant. For green, the values of r for the two samplings were -0.63 and -0.30. As the first of these exceeds the 1%, and the second attains the 5% point, both may be regarded as significant, and indicative of the fact that samples above average in initial green intensity tended to suffer a correspondingly greater reduction in this component of colour on exposure. A similar conclusion is to be drawn from the coefficients of -0.51 and -0.48 obtained from the measurements of blue intensity.

For sampling (iii), the observations in the red, green, and blue regions gave $r = -0.03$, $+0.36$, and $+0.50$ respectively over all samples, but these results were again clearly biased by batch differences, recomputations of the covariance about the batch means yielding $r = -0.31$, -0.42, and -0.39. Within batches therefore the indications are that, on the whole, greater decreases in each of the three component intensities occurred in those samples having the higher initial values.

Correlation with Chemical Analyses

In view of the anomalous circumstances attending sampling (iii), the correlation of the colour stability of individual sides with the results of chemical analyses was confined to samplings (i) and (ii). Even in these instances, it is necessary to take cognizance of possible complicating effects due to the association between the initial intensity of blue and green and the subsequent stability of these components, but it will be convenient first to examine the observed correlation between colour stability and the various chemical constituents studied, and then to determine whether any observed effects are to be regarded as due to the operation of the chemical factors directly upon stability, or indirectly through their influence on initial colour.

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN CHANGES IN COMPONENT COLOUR INTENSITIES
AND CHEMICAL ANALYSES OF BACON

Colour change	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
Sampling (i)	+.06	+.05	−.07	−.16	−.19
Sampling (ii)	+.17	−.11	+.38*	−.09	−.06
Both samplings	+.28*	+.03	+.17	−.21*	−.32**
Green intensity					
Sampling (i)	+.19	+.10	+.36*	+.32*	+.10
Sampling (ii)	+.10	+.09	+.26	+.08	+.01
Both samplings	+.22*	+.11	+.34**	+.16	−.05
Blue intensity					
Sampling (i)	−.04	−.02	+.22	+.25	+.20
Sampling (ii)	−.02	+.06	+.25	+.04	+.32*
Both samplings	+.09	+.02	+.26*	+.09	+.05

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Of the various simple correlation coefficients shown in Table V, those of nitrite content with green and blue stability alone exhibit any measure of persistency both within and between samplings. The apparent correlation in the data as a whole between red stability on the one hand, and salt content, pH, and moisture content on the other, is not in evidence within samplings, and hence may be due to differences in the means for the two samplings, rather than to an actual causal association. There is, however, some indication of an effect of nitrite on red stability in sampling (ii). In this, as in the other instances in which a significant effect of nitrite was demonstrable, an above average concentration of this substance was associated with increased colour stability.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN STABILITY OF COMPONENT COLOUR
INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated			Independent of		Correlation coefficient	
					Sampling (i)	Sampling (ii)
Change		*Analysis*	*Analysis*	*Change*		
Red	×	salt	Nitrate,	blue and green	+.02	+.15
			Nitrite,	blue and green	+.08	+.09
			Moisture,	blue and green	−.05	+.12
			pH,	blue and green	—	+.16
Red	×	nitrate	Salt,	blue and green	+.03	−.09
			Nitrite,	blue and green	—	−.05
			Moisture,	blue and green	−.03	—
Red	×	nitrite	Salt,	blue and green	−.12	+ 30
			Nitrate,	blue and green	—	+.32*
			pH,	blue and green	−.00	+.39*
Red	×	pH	Salt,	blue and green	—	−.07
			Nitrite,	blue and green	−.16	−.23
Red	×	moisture	Salt,	blue and green	−.14	−.09
			Nitrate,	blue and green	−.13	—
Green	×	salt	Nitrate,	blue and red	+.23	+.18
			Nitrite,	blue and red	+.13	+.14
			Moisture,	blue and red	+.30	+.01
			pH,	blue and red	—	+.19
Green	×	nitrate	Salt,	blue and red	+ 06	+.11
			Nitrite,	blue and red	—	+.09
			Moisture,	blue and red	+.15	—
Green	×	nitrite	Salt,	blue and red	+.22	+.08
			Nitrate,	blue and red	—	+.14
			pH,	blue and red	+.22	+.10
Green	×	pH	Salt,	blue and red	—	+.11
			Nitrite,		+.09	+.03
Green	×	moisture	Salt,	blue and red	+.17	−.30
			Nitrate,	blue and red	+.07	—
Blue	×	salt	Nitrate,	red and green	−.16	−.17
			Nitrite,	red and green	−.20	−.18
			Moisture,	red and green	−.10	+.06
			pH,	red and green	—	−.17
Blue	×	nitrate	Salt,	red and green	− 04	−.02
			Nitrite,	red and green	—	+.04
			Moisture,	red and green	−.01	—
Blue	×	nitrite	Salt,	red and green	+.09	+.08
			Nitrate,	red and green	—	+.04
			pH,	red and green	−.04	+.04
Blue	×	pH	Salt,	red and green	—	−.04
			Nitrite,	red and green	+.09	−.01
Blue	×	moisture	Salt,	red and green	+.07	+.45**
			Nitrate,	red and green	+.14	—

Exceeds 5% point (r = 0.31).
**Exceeds 1% point (r = 0.40).*

As was pointed out in the previously published discussion of initial colour (5), however, simple correlation coefficients may fail to provide an adequate representation of the actual underlying relations, owing to the mutual inter-correlation, in individual samples, of (i) certain of the chemical constituents (2), and (ii) of the green and blue stability, as well as (iii) of stability and initial intensity. The analysis of these relations was accordingly pursued further by the calculation of the third order partial correlation coefficients listed in Table VI. These provide a measure of the correlation between the concentration of each chemical and the stability of each component colour intensity, after making allowance for the interrelation of both quantities under consideration with the two other component intensities of colour, as well as with one additional chemical factor.

The results now suggest that the direct effect of nitrite content on colour stability was confined to the red region of the spectrum, and that the apparent correlation of this factor with green and blue stability, noted above (Table V), was due to the interrelations mentioned. The only other significant coefficient in Table VI is that between blue stability and moisture content, independent of red stability, green stability, and salt content, in sampling (ii). Salt content itself seems to have been without measurable effect on either colour or colour stability, and pH, which appeared to have a significant influence upon initial blue intensity, likewise seems to have been without effect on the subsequent stability of this component.

The foregoing inference respecting the influence of nitrite content on red change was strengthened by the calculation of the sixth order partial correlation coefficient between these quantities, independent of salt content, nitrate content, pH, initial red intensity, green change, and blue change, which was found to be 0.43. This is the more noteworthy, since the demonstrable effects of nitrite on initial colour were confined to the blue region (5). On the other hand, the apparent association between blue stability and moisture content noted above would seem to have been spurious, as the fourth order coefficient, independent of initial blue intensity, was reduced to the insignificant level of $r = -0.09$. The absence of correlation between green change and the chemical constituents studied was confirmed by the calculation of fourth order coefficients, independent of initial green intensity, which proved to be uniformly insignificant.

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CANADIAN WILTSHIRE BACON

X. DISTRIBUTION OF CHLORIDE[1]

BY W. H. WHITE[2] AND W. H. COOK[2]

Abstract

Highly significant differences in chloride content were observed at different positions across the ham of Wiltshire sides, the portion next to the tank pickle during cure usually containing the most chloride. Of the various factors studied, the number of injections used for pumping a side, and the age from cure, were the only ones found to affect the chloride distribution. Greater uniformity was obtained by increasing the number of injections, and with increasing age from cure. Within the range used in practice for curing Wiltshire sides, the chloride content of the pickles or the number of days in cure was not related to the chloride distribution. An equation is given relating the uniformity of chloride distribution to the number of injections used per side in pumping, and the number of days from the end of cure. This equation indicates that, on the average, the variations in the chloride content within the ham of a Wiltshire side will be reduced to the same magnitude as the variations between sides cured in the same plant, after holding at 1.1° C. for periods of about 12, 26, and 30 days, for sides receiving 27, 8, and 0 injections, respectively.

Introduction

Curing of bacon involves not only the addition of a sufficient quantity of curing salts, but also their distribution throughout the meat. The period of maturation, following removal of the bacon from cure, is believed to improve the quality of the product, particularly with respect to colour uniformity and stability, and the development of the typical bacon flavour. Because of the relatively short curing periods employed in the preparation of Wiltshire bacon, it seems probable that the distribution of salts takes place mainly after removal from cure. Presumably such distribution must reach a certain level of uniformity before the desirable changes involved in maturation can occur. The apparent importance of a uniform salt distribution, both from its direct effect on palatability and its possible indirect effect on maturation, led to a study of this subject on factory-cured Wiltshire bacon.

The content of curing salts and properties of Wiltshire bacon obtained from different Canadian packing plants, and the effect of storage and smoking on these quantities, have been reported in earlier papers of this series (5, 6). The present study was confined to the distribution of chloride throughout the ham

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at the different samplings, with a few additional determinations on the content and distribution of chloride, nitrate, and nitrite, in pumped and unpumped sides.

Since the sides obtained from different plants varied in age from two to 11 days from the end of cure when the first analyses were made, it is obvious that the results do not give a measure of the rate of salt penetration during cure.   In commercial practice, moreover, pickle is injected into Wiltshire sides at a number of points before they are placed in the curing tank.   This practice, termed pumping, doubtless tends to increase the total salt content and to improve the uniformity of salt distribution, but the variations between the methods employed in different plants could easily mask the effect of differential rates of salt penetration in different carcasses.   Factors involved in pumping include the amount of pickle injected, and the position and number of stitches used.   Of these quantities, only the effect of the number of stitches could be determined, since the position of the stitches cannot be described in quantitative terms, while the amount of pump pickle added to the sides in question was unknown.   It appears, however, that the quantity of pickle retained by the sides is a function of the number of stitches employed (4).

Material, Sampling, and Methods

A description of the sides used in this study, the method of transfer from the plants to the laboratory, and the conditions of storage between samplings, has been reported in the first paper of this series (7).   The samples were obtained in the conventional manner (1) from a central strip, about $1\frac{1}{2}$ in. wide, taken from the slice of ham removed for analysis at each sampling (7). These strips included the cross section of the femur, since certain practical operators attach considerable importance to the salt concentration of the meat in the vicinity of the bone.   There is also some evidence (3) that the adductor and vastus intermedius muscles do not absorb salt readily.   Each strip was divided into three, instead of the five or more sections usually employed in studies of this sort.   This practice was followed since the hams on Wiltshire sides are rather small and, being compressible, tend to vary somewhat in thickness, depending on the conditions and method of baling and transport. The position from which each of the samples was taken is shown in Fig. 1. The material from Position 1, on the inside of the ham, was adjacent to the tank pickle during cure; that from Position 2 represented the middle of the ham, surrounding the bone; while Position 3 represented the meat adjacent to the layer of fat and skin, through which little salt is likely to penetrate during cure.

The lean meat was separated from the bone, fat, and connective tissue, and each portion thoroughly mixed and ground by passing it through a food chopper.   The samples were then frozen and stored at $-29°$ C., until required for analysis.   The chloride content was determined according to the official method of the A.O.A.C. (9, p. 354), in which the sample is ashed, leached with hot water, and the chloride content of this extract found by the Volhard

FIG. 1.   *Portions of Wiltshire hams used for determining distribution of chloride.*

procedure.   Statistical methods (10) were employed for interpreting the results.

The entire group of 44 sides were analysed at the first and second samplings; i.e., when received at the laboratory, and again after 10 days' storage at $1.1° C$. Only 16 of these sides were analysed for chloride distribution at the third sampling, i.e., after smoking.   While in subsequent tables the results for the entire group are considered together for the first two samplings, those for the 16 sides analysed three times are also considered separately, in order to provide comparable results over all three samplings.

Mean Chloride Content by Positions and Samplings

The mean age and sodium chloride content by positions and samplings over all sides are given in Table I.   The chloride content of the slice of ham from which the chloride distribution samples were taken has already been reported (6).   The table is divided into two main sections, the first giving the results for the 44 sides analysed twice and the second the results of the 16 sides analysed three times.

Although the mean age of the entire group of sides from cure was about seven days at the time of the first sampling, it is evident that the sodium chloride content at Position 1, next to the tank pickle, was much higher than at Positions 2 and 3.   This gradient still existed, but was much smaller, at the second sampling when the sides were about 18 days old.   The group of 16 sides analysed three times indicated essentially the same behaviour at the first two samplings, and also showed that the chloride is quite uniformly distributed after smoking, when the mean age for the group was about 24 days.

The results reported in the last column represent the best estimate of the true chloride content of the hams, since these figures (6) were obtained by grinding and sampling a piece of each ham several times as large as that of the entire strip used in the salt distribution studies.   Other investigators (8) have observed that this central-strip sampling procedure does not represent

TABLE I

MEAN SODIUM CHLORIDE CONTENT OF SIDES BY POSITION AND SAMPLING

Sampling	Mean age of sides from cure, days	Position	Mean sodium chloride content		
			By position, %	By sampling, %	From previous analyses (6), %
For all 44 sides—sampled twice					
First	7.4	1 2 3	6.42 3.45 3.03	4.30	3.43
Second	17.8	1 2 3	4.49 3.78 3.46	3.91	4.42
For 16 sides—sampled three times					
First	9.9	1 2 3	6.87 3.47 2.69	4.34	3.19
Second	21.4	1 2 3	4.46 3.73 3.33	3.84	4.19
Third	24.4	1 2 3	4.06 3.90 4.01	3.99	—

the true salt content of the ham.   The present results indicate that the mean chloride content over all three positions in this central strip apparently over-estimates the true chloride content at the first sampling and underestimates it at the second.   In more specific terms the chloride content at Position 1 was much above the average at the first sampling, while that at Positions 2 and 3 was still below the average at the second sampling.   From this it appears that the positions chosen represent not only those likely to have the highest chloride content initially, but also those that are the last to be affected by the movement of chloride throughout the ham.

The significance of the observed differences in the chloride content between positions at the different samplings was determined by an analysis of the variance for each sampling, into portions attributable to: between positions; between sides from different plants; between sides from the same plant; the interaction positions × plants; and sampling and analytical error.   The results, which appear in Table II, show that the difference between sides from different plants is usually significantly greater than the differences between sides from the same plant, and that this in turn usually exceeds the sampling and analytical errors.   This merely confirms the findings reported

TABLE II

ANALYSIS OF VARIANCE OF SODIUM CHLORIDE CONTENT AT DIFFERENT SAMPLINGS

	Sampling					
Variance attributable to	First		Second		Third	
	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
For all 44 sides—sampled twice						
Between positions	2	149.926**	2	12.206**	—	—
Between plants	21	4.148	21	3.320**	—	—
Between sides	22	2.202**	22	0.797**	—	—
Differential effect, position × plants	42	1.977**	42	0.433**	—	—
Sampling and analytical error	44	0 674	44	0.177	—	—
For 16 sides sampled for salt distribution—three times						
Between positions	2	79.186**	2	5.262**	2	0.102
Between plants	7	8.129**	7	5.227**	7	7.329**
Between sides	8	0.798**	8	0.583	8	0.844
Differential effect, position × plants	14	1.232**	14	0.343	14	0.250
Sampling and analytical error	16	0.175	16	0.287	16	0.434

* *Indicates 5% level of significance.*
** *Indicates 1% level of significance.*

in an earlier paper (6). The fact that these differences were sometimes not significant at certain samplings in the present study does not indicate disagreement with the earlier results, since they dealt with the average effect over all sides and samplings.

The present study is concerned primarily with the variance between positions, since this quantity reflects the variations in chloride distribution. It is evident that the observed differences in the chloride content at different positions, as indicated by the results in Table I, are statistically significant at the first two samplings. Since the mean square between positions decreases between successive samplings, it is evident that the chloride becomes more uniformly distributed during the maturation period. In the group of sides sampled after smoking, the difference between positions is not significant, indicating the absence of a systematic gradient in chloride concentration.

The interaction position × plants was also significant at both samplings for the entire lot of 44 sides. This indicates that there was not a uniform gradient in chloride concentration from one position to another in the product of all plants. It is possible that this could be attributed to the different pumping practices, etc., followed in different plants. Since the sides from the different plants varied in age from two to 11 days from cure at the time

of the first sampling, it may be that the different gradients merely reflect the effect of ageing.

Chloride Distribution in Relation to Curing Practice

Before attempting to establish a quantitative relation between the chloride distribution and the age of the sides, some consideration had to be given to the effect of variations in the curing practices, in order that these factors could be taken into account. The standard deviation between the positions for both sides received from each plant was used as an estimate of chloride distribution. Although the standard deviations between all three positions were used initially, it was found, as the computations proceeded, that the difference between Positions 1 and 3 was an equally sensitive measure of the variations in chloride content. The standard deviation or difference between the positions observed for all sides at the first sampling was correlated with the known curing factors, as outlined in the first part of Table III. The results of the second sampling were not included, since the influence of age tends to minimize any difference attributable to the various curing practices.

Examination of these correlation coefficients shows that only the coefficient relating the standard deviation between positions and the number of stitches used per side attains the 5% level of significance. In fact, the other coefficients relating quantities involving the number of stitches per side, although not significant, were much larger than the correlations between the standard deviation and other quantities, and probably would have been significant had the sides been analysed immediately after cure. It is therefore concluded that chloride distribution is influenced more by the number of stitches used in pumping than by the observed variations in the chloride content of either of the pickles or by the number of days in cure.

In an earlier paper (6), it was shown that the number of stitches injected per side was the only known curing practice that was correlated with the over-all chloride content of bacon. Since the chloride content at Position 1 should be affected primarily by the tank curing practice, and that at Positions 2 and 3 by the pumping practice, correlation coefficients were computed between the chloride contents at these positions, for the first sampling, and the known properties of the pumping and tank curing practices. The results obtained are shown in the second part of Table III. Although none of the correlation coefficients are significant, their magnitude suggests that the number of stitches per side is the most influential factor of those studied in determining the chloride content at Position 2. It appears, therefore, that within the limits used in practice, similar amounts of chloride are taken up from the tank pickle regardless of its concentration or period in cure. In these circumstances, pumping the sides would be expected not only to reduce the variation in chloride content between the outside and the centre positions, but also to increase the total amount in the bacon. Nevertheless, correlation coefficients failed to demonstrate a definite relation between chloride content and chloride distribution.

TABLE III

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE DISTRIBUTION
AND CERTAIN CURING PRACTICES

Quantities correlated	D.F.	r
Sodium chloride distribution in relation to curing practice		
Standard deviation of sodium chloride content at all 3 positions with:		
No. of stitches per side	20	−0.42*
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
Difference in sodium chloride content between Positions 1 and 3 with:		
No. of stitches per side	20	−0.42*
Days in cure	20	−0.14
Sodium chloride in tank pickle	20	+0.01
No. of stitches per side × sodium chloride in pump pickle	20	−0.38
No. of stitches per side, independent of days in cure	19	−0.41
No. of days in cure, independent of number of stitches per side	19	−0.08
Sodium chloride content in relation to curing practice		
Sodium chloride content of meat at Position No. 1 with:		
Days in cure	19	+0.14
Sodium chloride in tank pickle	19	+0.01
Sodium chloride in tank pickle × days in cure	19	+0 14
Sodium chloride content of meat at Position No. 2 with:		
No. of stitches per side	20	+0 37
No. of stitches per side × sodium chloride in pump pickle	19	+0 34
Sodium chloride content of meat at Position No. 3 with:		
No. of stitches per side × sodium chloride in pump pickle	19	+0.07
Relation between distribution and content of sodium chloride		
Standard deviation in sodium chloride content at all 3 positions with mean chloride content of all 3 positions for:		
First sampling by plants	20	−0.18
First sampling by sides	42	−0.11
Second sampling by plants	20	−0.36
Second sampling by sides	42	−0 29

Indicates 5% level of significance.

Comparison of Pumped and Unpumped Sides

The results presented in this and earlier papers (5, 6) have indicated the importance of the method of pumping, in determining the concentration of curing salts in bacon. To confirm this a few preliminary experiments were made on the composition of pumped and unpumped sides cured simultaneously in the same tank in a packing plant. Four pumped and two unpumped sides were sampled and analysed, by the methods previously described (11), immediately on removal from cure and again 17 days later. The mean com-

position of each group of sides, with respect to sodium chloride, nitrate, and nitrite, appears in the first part of Table IV, and the chloride distribution, as shown by the sodium chloride content at the different positions, in the second part. Statistical methods were not applied to these few results, but since the different sides in each group varied considerably, only relatively large differences can be considered significant.

TABLE IV

COMPOSITION OF, AND CHLORIDE DISTRIBUTION IN, PUMPED AND UNPUMPED SIDES

Constituent	Pumped		Unpumped	
	1st sampling	2nd sampling	1st sampling	2nd sampling
Constituent—				
Sodium chloride, %	2.61	3.68	0.56	1.93
Sodium nitrate, %	0.16	0.16	0.008	0.028
Sodium nitrite, p.p.m.	17	34	0.8	27
Sodium chloride distribution				
Position—				
1	4.92	2.96	3.15	2.28
2	3.08	2.91	0.82	1.26
3	3.41	3.48	0.46	1.05

It is evident that the slice of ham analysed at the first sampling contained less of all three salts than at the second sampling. In general, this apparent increase in the content of the curing salts must be attributed to differences between positions rather than a change with time (6). Nevertheless, the proportionately greater increase in the nitrite content of the unpumped sides between samplings suggested a real change during storage. This confirms an earlier finding (6) that nitrite is produced in sides having a low nitrite content initially.

Comparison of the values obtained at the second sampling, when a reasonably uniform distribution of the salts has occurred, indicates that pumping contributes about half the chloride and 80% of the nitrate found in the bacon. The chloride content at the three positions for the second samplings suggests a relatively uniform distribution throughout the hams of the pumped sides, while the unpumped sides still had about twice as much chloride at the position adjacent to the tank pickle, as at the central positions. The importance of pumping in determining both the concentration of the salts and their distribution in the sides is apparent.

Effect of Pumping and Ageing

It was concluded from the results already presented that the number of stitches used for pumping and the age from cure were the principal factors affecting the distribution of salts in hams of Wiltshire sides. In order to obtain

a quantitative estimate of the effect of these two factors, a partial regression equation was computed for the standard deviation between positions on number of stitches used in pumping and the number of days from cure over all samplings. This equation was found to be:

$$Y = 4.64 - 0.074\,S - 0.126\,N$$

where

Y = standard deviation between salt content at different positions,

S = number of stitches used per side

and

N = number of days from cure.

Although this equation shows the extent to which the variations in chloride content between different positions decrease with increase in number of stitches per side and days from cure, it is also important to determine the amount of the total variance accounted for in this way. The mean square for the observed variations in Y (standard deviation between positions) was found to be 1.42, and after subtracting the variance accounted for by the above equation, the residual mean square was 0.70. This shows that variations in pumping practice and age of the sides accounted for about half of the observed variance.

From a practical standpoint, there is no apparent advantage in reducing the variations in salt content within an individual side to less than those between different sides produced under the same conditions in the same plant. Reference to Table II shows that the mean square between sides over all samples at the second sampling was 0.80.
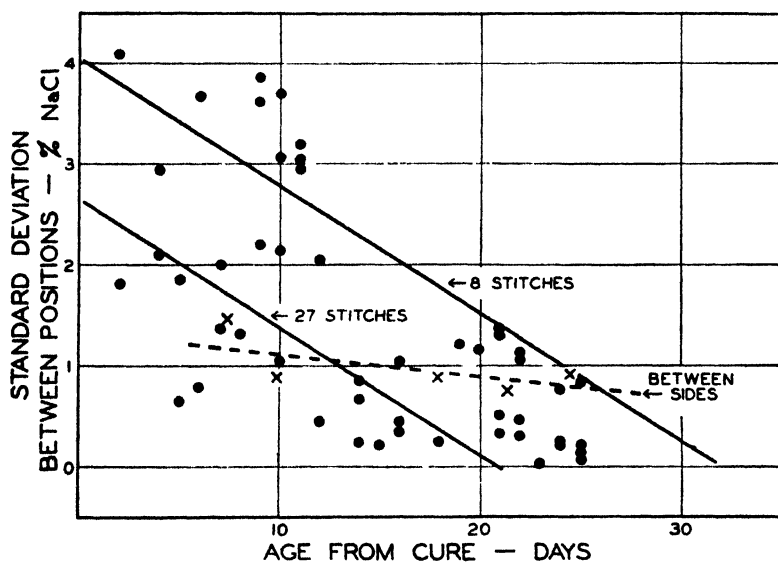

FIG. 2. *Effect of number of stitches used in pumping, and the age of the side on the distribution of chloride.*

Using this criterion, it is possible to predict the time required, following various pumping practices, to obtain a satisfactory chloride distribution throughout the ham of a Wiltshire side.   In order to do this the equation given earlier was used to construct Fig. 2, in which the estimated standard deviation between positions is plotted against the age of the sides from cure. The upper line represents the results with the minimum (eight), and the lower, with the maximum (i.e., 27) number of stitches used for pumping the sides studied in this investigation.   The experimental points, representing the mean value for the two sides received from each plant, are plotted for comparison. The crosses and broken line indicate the level of the standard deviation between sides, computed from the mean square given in Table II, for sides of various ages from cure.   The time required for the standard deviation between positions to fall to this level represents that required to obtain a satisfactory chloride distribution.   The figure suggests that this level could be attained in about 12 and 26 days, respectively, for sides receiving the maximum and minimum number of stitches ordinarily used in pumping.   Since Canadian sides never arrive in England in less than 12, and on the average 18, days after removal from cure, it appears that they must be satisfactory from the standpoint of chloride distribution before they reach the consumer.

Discussion

Considerable work has been done on the rate of salt penetration into meat, and its subsequent distribution within the cured product.   An extensive review of the literature on this subject is unnecessary, but it is of interest to consider the findings of certain investigators in relation to those obtained in this study.   Reduced to essentials, the present results show that the number of stitches used in pumping and the age of the sides from cure were the only factors of those studied found to affect the chloride distribution in Wiltshire bacon as manufactured by current methods.

Banfield and Callow (2) have shown that the decrease in electrical resistance during cure was less marked in unpumped than in pumped sides.   This implies that the unpumped sides contained less salt, a result confirmed by analysis after maturation, although the small difference observed for tank cured sides is doubtless of little practical consequence.   These investigators used the backs, and since the lean portion at this point is smaller than that of the ham, a more uniform penetration and distribution of the chloride is to be expected, and the effect of pumping consequently less marked.

Besley and Hiner (3) have shown that the various muscles in unpumped lamb legs absorb salt differentially, and claim that even larger differences occur between the different muscles in hams.   The results of the present study suggest that where a reasonably satisfactory pumping practice is used, with respect to both the number and position of the injections, the differential absorption of chloride by different muscles would have little effect on the chloride distribution.   They also found that the number of days in cure was the principal factor affecting the chloride content and distribution between different

muscles. Since these lamb legs were both unpumped and cured for a period far in excess of that generally used for curing Wiltshire bacon, these findings do not appear to contradict those obtained in these investigations (3).

Miller and Ziegler (8) studied the chloride distribution in hams, using sampling and statistical methods comparable to those used in this study. The hams, however, were not pumped and the length of the curing period was considerably longer than that used for Wiltshire sides. Their results indicate that following a brine-curing period of 30 days, a holding period of another 30 days at 37° F. was required to reduce the coefficient of variability between positions to about 20% or less. This variability corresponds to a standard deviation of about 0.80% when interpreted in terms of the mean sodium chloride content, expressed on a "wet" basis, as observed in these studies and reported in Table I and previously (6). Using the equation given earlier, with $S = 0$, since no pickle was injected, and $Y = 0.8$, a value of about 30 is obtained for N. It is indeed surprising that the results of these two independent investigations agree so closely, considering that they represent work with pumped sides and unpumped hams, cured for widely different periods, and subsequently stored at somewhat different temperatures.

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CANADIAN WILTSHIRE BACON

XI. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT[1]

By W. H. White[2], W. H. Cook[2], and C. A. Winkler[3]

Abstract

Small cuts of matured Wiltshire bacon were held at temperatures of 20, 40, 50, 60, 70, and 80° C. for periods of 5, 10, 20, and 40 hours. When the nitrite content after treatment was plotted against the temperature, the results showed that the highest nitrite contents were obtained at 40° C., the nitrite content decreased logarithmically between 40 and 80° C., and the slope of these curves increased with increasing periods of treatment. The curves representing the different periods of treatment intersect in the region of 55° C., where the nitrite content was approximately normal. It appears, therefore, that an increase in nitrite content above the normal level occurred at temperatures below 55° C. and a decrease at higher temperatures. This decrease in nitrite nitrogen above 55° C. may be attributed to direct loss, oxidation, or reaction with the constituents of the meat. The increase in nitrite observed at temperatures below 55° C. suggests that time and temperature were not alone responsible for the observed changes, since comparable increases in nitrite were not observed in commercial cuts. It may be that the proportionately greater surface exposed to the air in these small samples may have had some effect, such as an enhanced aerobic bacterial action.

Introduction

The characteristic colour of bacon is attributed primarily to the combination of nitrite with the muscle haemoglobin to form nitrosohaemoglobin. This compound is believed to be converted to a more stable form, termed nitrosohaemochromogen, when the meat is cooked, and this change may also occur to some extent during smoking at higher temperatures, i.e., above about 55° C. In earlier papers (7, 8) of this series, it has been shown that the pH and nitrite content of the bacon affected colour and colour stability. It may be that these influential factors act either by determining the rate or extent of the reaction producing nitrosohaemoglobin, or by an independent effect on the colour of the bacon. The nitrite content of bacon may not reflect its nitrosohaemoglobin content, but as a factor affecting colour it deserves consideration. It has also been shown (3, 6) that the nitrite content of certain sides, through bacterial action or otherwise, may increase during holding after cure, and decrease slightly during smoking at about 45° C. (3).

An investigation was, therefore, undertaken to determine the effect of heating Wiltshire bacon, for various periods at different temperatures, on its nitrite content and colour. This paper deals with the changes in nitrite content.

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Material, Design, and Method

Since the earlier studies (3) showed that different sides, treated in the same manner, differed significantly in nitrite content, it seemed desirable to have different sides cured in several different plants represented in the experiment. The available material consisted of "backs", cut from two sides (from different pigs) cured in each of eight different packing plants. These sides represented bacon of various ages, having been removed from cure and held at temperatures of 1 to 3° C. for periods of from 23 to 25 days before being sampled for these studies.

The experiment was arranged in a factorial design (4), involving: 4 times of treatment, namely, 5, 10, 20, and 40 hr.; 6 temperatures, namely 20, 40, 50, 60, 70, and 80° C.; and 4 replications of each treatment. The 96 pieces required were obtained as follows: The 16 backs were randomized over the 4 replicates and 4 times of treatment; each back was then divided into 6 approximately equal parts of about 100 to 200 gm. each, and these pieces were randomized among the 6 temperatures within times. It is obvious that this design yields more precise information on the effect of temperature than of time, since the significance of the observed differences with temperature is tested by comparison with the analytical and sampling error within sides, while the differences between times of treatment must be tested by comparison with the difference between sides. Within the limitations of available material, time, and facilities, it seemed more desirable to obtain precise information on the effect of temperature.

The samples were first trimmed free of the cut portions of the ribs and the layer of back fat. The individual pieces were weighed, wrapped in lightly waxed glassine, and then in kraft paper. The individual packages to be heated at a given temperature were wrapped into a single package. These operations were performed in a laboratory at about 25° C. The samples were placed in ovens at the required temperatures, removed after the required period of time, and weighed. The surface layers were trimmed off, suitable samples taken for colour measurements, and the remainder, trimmed free of fat and connective tissue, was ground and mixed by several passages through a food chopper, bottled, and frozen at −29° C. The samples were thawed as required, analysed by the method previously described (5), and the results treated by statistical methods (4).

Results

The loss of weight during heating was a function of the time and temperature of treatment. At the higher temperatures these losses represented not only a loss of moisture but also of a certain amount of fat that melted and was absorbed by, or lost from, the paper wrapping. These figures were used for computing the amount of nitrite in terms of the original sample weight, but otherwise are of little significance in the present study.

The mean nitrite content of the four samples treated under each condition is reported in Table I.   In considering these results, it must be remembered that there was considerable variation between the individual pieces from different sides treated in the same way.   This, together with the fact that the nitrite content after the different treatments also varied widely, made it necessary to employ statistical methods to test the significance of the observed differences.

TABLE I

MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS

(As p.p.m. in initial weight)

Period of heat treatment, hr.	Temperature of heat treatment, °C.					
	20*	40	50	60	70	80
5	25.81	22.16	19.73	16.94	20.16	14.38
10	29.21	41.88	41.40	15.02	8.62	5.49
20	179.52	191.25	51.35	10.20	5.76	2.36
40	—	404.75	33.95	9.41	3.82	1.32

* Samples treated at this temperature considered separately in subsequent statistical analysis.

The values reported in Table I show that the samples varied in nitrite content from about one to over 400 p.p.m.   The deviations of the individual values from the reported means were approximately proportional to the mean.   Since results exhibiting a variability of this magnitude cannot be used directly for an analysis of variance (2), they were first converted into logarithms of the nitrite content in parts per million before the statistical computations were undertaken.   All numerical values subsequently reported are on this basis.

The samples held at 20° C. were used to determine the changes in nitrite content that occur at room temperature.   Unfortunately, the samples that were to be held for 40 hr. at this temperature were removed and ground after 20 hr., so that 8 samples were treated for 20 hr. at this temperature but none for 40 hr.   This loss was not serious, however, since it seemed desirable, in making the statistical analyses, to treat the results obtained at 20° C. separately from those obtained at higher temperatures.

The results of an analysis of variance are reported in Table II.   It is evident that the difference between the nitrite content of the samples held at 20° C. is highly significant.   This shows that the increase in nitrite content, as shown in Table I,   exceeds the variations observed between different sides, even within a 20-hr. period.   The remaining samples, treated at higher temperatures, were considered together, and for these the average change in nitrite content with time is not significantly greater than the differences between the different sides that were treated similarly, but is significantly greater than the sampling and analytical error within sides.   From

TABLE II

ANALYSIS OF VARIANCE.   EFFECT OF HEAT TREATMENT ON NITRITE CONTENT
OF BACON

(Values in terms of logarithms of nitrite content in p.p.m.)

Variance attributable to	Degrees of freedom	Mean square
Samples heated at 20° C.		
Between times of treatment	2	1.426**
Between sides within times	13	0.124
Samples heated at 40 to 80° C.		
Between times of treatment	3	0 491
Between sides within times	12	0.509
Between treatment temperatures	4	4.812**
Differential effect of temperature × time	12	0.597**
Sampling and analytical error within sides	48	0.035

** *Indicates 1% level of significance.*

this it is concluded that variations in the properties of the individual piece or side, or in their bacterial content or flora, determine, to a large extent, the concentration of nitrite present after exposure to heat treatments comparable to that of smoking.   This conclusion is in general agreement with results reported previously (3, 6).

The differences between the nitrite content following a given period of treatment at different temperatures of treatment, can be determined more exactly, since the same sides were represented at all temperatures.   It is evident from Table II that the differences between temperatures and the variance attributable to the interaction, temperature × time, were both highly significant.   This means that not only is the nitrite content a function of the temperature of treatment, but also that temperature has a differential effect on the nitrite content, depending upon the duration of treatment.

The direction of these significant changes in nitrite content under different conditions is evident from the results reported in Table I, but is shown more clearly in graphical form in Fig. 1.   In this figure the nitrite content is plotted on a logarithmic scale against the temperature of the treatment.   The four curves shown represent the different periods of treatment.   Since the results obtained at 20° C. were considered separately in the statistical analyses, and since nothing is known of the changes that occur in the temperature range from 20° to 40° C., the points obtained at these two temperatures have been joined by a broken line, although the nitrite-temperature relation is doubtless curved in this range.
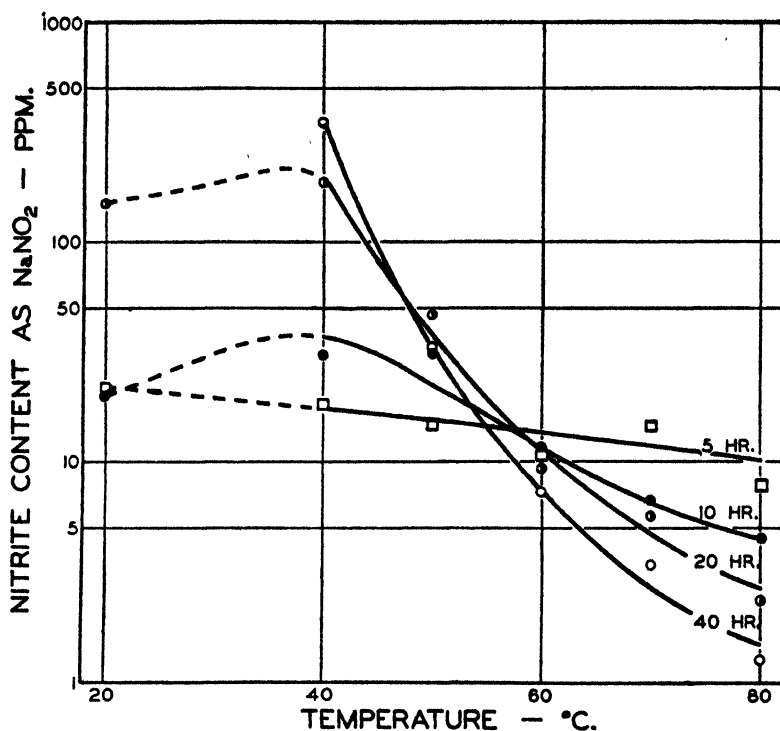
FIG. 1.   *Effect of time and temperature of heating on the nitrite content of Wiltshire bacon.*

It would appear that the nitrite content, following a given period of treatment, is generally higher at 40° C. than at 20° C. Above 40° C. the nitrite content falls off more or less logarithmically as the temperature increases. Extending the period of treatment increases the nitrite content at 40° C., and decreases it at 80° C. The curves representing the various periods of heating, therefore, differ significantly in slope (Table II), and intersect one another in the region of 55° C., where the nitrite contents were approximately normal.

Discussion

Callow (1) has found that the nitrite content of small cuts of bacon may increase during storage for several weeks after curing. He found that the maximum nitrite concentration occurred at 5° C. and attributed the increase to bacterial action. The experiments reported in this paper were made over shorter periods at higher temperatures. In the present investigation the highest nitrite concentration occurred at 40° C., although the results at 20° C. suggest the possibility of a maximum nitrite production between 20 and 40° C. Since the optimum temperature for most bacteria lies between 20 and 40° C., it would seem reasonable to expect, if nitrite production is dependent on microbial activity, the maximum production of nitrite in this temperature range, rather than at cellar temperature.

In the main series of experiments (3), involving whole sides or large cuts, comparable increases in nitrite content were never observed, even in an individual side, during 10 days' storage at 1.1° C., smoking overnight at 40 to 45° C., and holding at room temperature for periods of 12 to 24 hr. In fact, smoking under these conditions tended to reduce rather than increase the nitrite content. These results suggest that time and temperature of treatment were not alone responsible for the increase in nitrite nitrogen observed in the present study.

It is possible that the proportionately greater surface exposed to the air in these small cuts may have been responsible for the observed behaviour. Certain direct evidence favouring this hypothesis was obtained from the observation that ground bacon stored at 4° C. showed a large increase in nitrite content within 36 hr., while the same bacon in the whole side showed no significant change. It appears, therefore, that the present results represent the effect of time and temperature under conditions that probably exaggerate their influence on nitrite production, as compared with those existing in commercial practice.

Although the results indicate that the nitrite content increased at temperatures below about 55° C., the agency responsible for these changes cannot be determined from these experiments. The accelerating effect of exposure to the atmosphere suggests that aerobic bacteria are responsible for nitrite production, but it is also possible that certain constituents or enzymes of the meat are capable of reducing the nitrate present to nitrite. Further work has been undertaken to determine the factors responsible for the observed increase in nitrite concentration.

Since protein coagulation occurs at temperatures in the vicinity of 55° C., bacterial and enzymic activity would be reduced to negligible proportions by exposure to higher temperatures. At temperatures above 55° C., the observed decrease in nitrite content may be attributed to chemical reactions causing a direct loss, oxidation, or combination of nitrite with the constituents of the meat.

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Effectiveness of Ice containing Sodium Nitrite for Fish Preservation

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ABSTRACT

Bacterial spoilage of dressed halibut, pink salmon and black cod is markedly inhibited by icing with crushed ice containing small amounts (0.1 or 0.5 per cent) of sodium nitrite instead of ordinary ice, as demonstrated by bacteriological examination and organoleptic tests, including appearance (halibut excepted), odour and taste. Halibut becomes a marked yellow on the ventral (white) surface. Sodium nitrite ice preserves fish much more efficiently than benzoic acid ice.

In a previous communication (Tarr and Bailey 1939) it was shown that ice containing 0.1 per cent of benzoic acid exerted very little beneficial effect on the keeping quality of black cod and halibut, as judged by viable bacterial counts and trimethylamine content of the muscle. Hjorth-Hansen and Karlsen (1939) have since shown that ice containing 0.012 to 0.015 per cent of hydrogen peroxide effects only a slight improvement in the keeping quality of dressed fish and fillets. The fact that nitrites are much more effective than benzoates for preserving both fresh and lightly smoked fillets (Tarr and Sunderland 1939a, 1939b; 1940) suggested the possibility of employing ice containing nitrite to preserve dressed fish. The results of experiments designed to test this point have already been briefly described (Tarr and Sunderland 1939c), and are recorded in detail herewith.

METHODS

While benzoic acid ice (Bedford unpub.) can readily be prepared as a true eutectic compound from an aqueous solution containing approximately 0.156 per cent of the chemical, sodium nitrite, when used in similar amounts naturally tends to become concentrated in the liquid phase during the freezing process, preventing uniform distribution. However, experiments have shown that a considerable proportion of the sodium nitrite is scattered mechanically (occluded) throughout the mass, as has been found to be the case with ice made from weak hydrogen peroxide solutions (Hjorth-Hansen and Karlsen 1939). Just as agitation of water during freezing facilitates the concentration of impurities in the centre "core" of the ice blocks, so does aeration concentrate the sodium nitrite, as is evident in table I. It is therefore obvious that in preparing sodium nitrite ice the water containing this salt should not be aerated (stirred) during freezing, and that the cores of the resulting ice blocks should not be "drawn" and refilled

36

with water as is customary. When blocks of ice containing sodium nitrite are crushed preparatory to use, a fairly uniform distribution of the salt throughout the finely divided particles results (table I).

TABLE I. Distribution of sodium nitrite in 400 lb. (182 kg.) blocks of ice* prepared from aerated and non-aerated 0.05 per cent (500 parts per million) aqueous solutions of the salt.

Region of block sampled	NaNO₂ in parts per million ,	
	Aerated during freezing	Not aerated during freezing
Surface sample, not over 2 cm. deep, about 3 cm. from base of block.................................	28	408
Surface sample, not over 2 cm. deep, about half way from base	12	392
Top corner, not over 5 cm. deep	6	500
Core, about 5-7 cm. diameter, 1/3 way from base	7500	1480
Core, about 5-7 cm. diameter, 2/3 way from base	8000	2420
Core, about 5-7 cm. diameter, at top of block.............	6500	1290
About 3 kg. of melted ice after crushing and mixing thoroughly	490	512

*Frozen in vertical tanks in brine for about 2 days at 10°F.

Samples for determination of the viable bacterial population of the fish muscle were taken from the poke region in the case of halibut, and from one side of the fish in the case of pink salmon and black cod, the technique of making bacterial counts and excising the muscle being identical with that previously described (Tarr and Bailey 1939). Organoleptic tests were made by the writers, and included the external appearance of the iced fish, the odour of the poke, and the taste of the flesh ("belly muscle" in the case of halibut) after cooking for 10 minutes at about 300°C. in an electric oven. The amount of sodium nitrite in treated fish muscle was determined by the method described elsewhere (Tarr and Sunderland 1940).

EXPERIMENTS

EXPERIMENT 1.

Twelve halibut (*Hippoglossus stenolepis*), weighing from about 5 to 20 lb. (2 to 9 kg.), were dressed and the pokes scraped and washed with sea water in the usual manner on board a fishing boat soon after capture. Six fish were iced with ice containing 0.5 per cent sodium nitrite and six with tapwater ice. They were landed after 2 days, re-iced with their respective ices, and were stored in boxes in the ice room of a local fish company, fresh ice being added at intervals to replace that which had melted. Individual fish were removed from time to time in order to determine the extent of spoilage. The results are recorded in table II.

EXPERIMENT 2.

Ten halibut weighing from about 8 to 10 lb. (3.5 to 4.5 kg.) were obtained from a local fish company. These fish had been dressed, washed and iced on

TABLE II. The influence of ice containing 0.5 per cent sodium nitrite on the keeping quality of halibut.

Age of fish in days after capture	Count of viable bacteria in poke muscle (colonies per g.)		Results of organoleptic tests*		NaNO₂ in p.p.m. of wet muscle in fish stored in ice containing nitrite	
	Tapwater ice	Ice containing NaNO₂	Fish from tapwater ice	Fish from ice containing nitrite	Poke muscle**	Belly muscle**
8	2,400	80	No noticeable difference in odour of pokes nor in flavour of cooked flesh		....	....
12	142,000	8,420	Odour of poke: "Sour"	"Fresh"	880	323
16	980,000	480	Odour of poke: Very sour. No difference in flavour observed on cooking	Not unpleasant, apparently fresh.	855	500
19	1,560,000	113	Putrid odour, fish covered with brownish-yellow slime. No difference in flavour of cooked fish	Odour not unpleasant, faint smell resembling nitric oxide.	....	....
26	13,800,000	2,520	Fish very putrid, surface covered with brownish slime. Cooked flesh: Stale and inedible	Odour not unpleasant, faint smell resembling nitric oxide. Edible and apparently fresh	....	....
31	10,480,000	46,000	Fish very putrid. Cooked flesh: Slightly stale, rather "tasteless".	Odour not unpleasant, faint smell resembling nitric oxide. Edible, but dry and rather tasteless	970	....

*Halibut stored in nitrite ice invariably develop a yellow discolouration. A piece of muscle about midway along the white (ventral) side opposite the poke was used in tasting.

**These terms were described in a previous paper (Tarr and Bailey 1939).

board a fishing boat and were landed 5 to 6 days after catching. Five fish were re-iced with ice containing 0.1 per cent sodium nitrite and five with tapwater ice. They were stored and re-iced as in Experiment 1, individual fish being examined at intervals (table III).

EXPERIMENT 3.

Pink salmon (*Oncorhynchus gorbuscha*) weighing 4 to 5 lb. (about 2 kg.) which had been stored, without being gutted, for about 2 days in ice after capture, were

TABLE III. The influence of ice containing 0.1 per cent sodium nitrite on the keeping, quality of halibut.

No. of days fish stored in the experimental ices	Count of viable bacteria in muscle (colonies per g.)		Results of organoleptic tests*		NaNO₂ in p.p.m. of wet muscle in fish stored in ice containing nitrite	
	Tapwater ice	Ice containing NaNO₂	Fish from tapwater ice	Fish from ice containing nitrite	Poke muscle	Belly muscle
9	1,320,000	288	External appearance: 　Good　　　　　　Good Odour of poke: Sour　Fresh No difference in flavour observed on cooking		350	....
13	6,800,000	24,800	External appearance: 　Fair　　　　　　Good Odour of poke: 　Very sour　　　　Fresh No difference in flavour observed on cooking		370	....
17	15,800,000	122,000	Odour of poke: 　Very putrid　　Not unpleasant No difference in flavour observed on cooking		258	65
20	6,380,000	474,000	External appearance: 　Poor, fish covered　Good 　with brownish-yel- 　low slime Odour of poke: Very　Not unpleasant 　putrid Cooked flesh: Stale　Good condition, edi- 　and inedible　　　ble		262	74
22	520,000	118,000	Appearance and odour of the two fish examined about the same as after 20 days Cooked flesh: Edi-　Edible, good condi- ble, good condition　tion		134	....

*See footnote, Table II.

used. Five fish were iced with ice containing 0.1 per cent of sodium nitrite and five with tapwater ice. They were stored, re-iced and examined as in the previous experiments, with the results recorded in table IV.

EXPERIMENT 4.

Ten black cod (*Anoplopoma fimbria*) weighing from about 4 to 5 lb. (2 kg.) were selected from a load of fish which had been 5 to 7 days in ice after dressing on board a fishing boat. Half the fish were iced with ice containing 0.1 per cent of sodium nitrite, and the remainder with tapwater ice. They were stored in a room having a temperature of 1.5 to 4°C., and were examined as in previous experiments (table V).

TABLE IV. The influence of ice containing 0.1 per cent of sodium nitrite on the keeping quality of pink salmon.

No. of days fish stored in the experimental ices	Count of viable bacteria in muscle (colonies per g.)		Results of organoleptic tests		NaNO₂ in p.p.m. of wet muscle in fish stored in ice containing nitrite. Muscle from one side of fish sampled as in making bacterial counts
	Tapwater ice	Ice containing NaNO₂	Fish from tapwater ice	Fish from ice containing nitrite	
9	7,200	264	No difference in odour, external appearance or flavour observed.		267
13	178,000	12,800	No noticeable difference in external appearance. Odour of poke: Sour Cooked flesh: Slightly stale	Fresh Fresh	89
17	1,180,000	154,000	External appearance: Very slimy, stale Odour of poke: Very stale Cooked flesh: Stale, almost inedible	Good Fresh Edible, good condition	
20	740,000	780	External appearance: Covered with brownish-yellow slime Odour of poke: Putrid Cooked flesh: Stale and inedible	Good No unpleasant odour Edible but tasteless	302
22	3,444,000	8,400	External appearance: Covered with brownish-yellow slime Odour of poke: Very putrid Cooked flesh: Stale and inedible	Fair No unpleasant odour Edible but tasteless	148

DISCUSSION

That ice containing 0.1 per cent of sodium nitrite is much more effective in inhibiting the growth of bacteria on dressed fish than is ice containing an identical proportion of benzoic acid is evident when the results of experiments described in this paper are compared with those previously recorded (Tarr and Bailey 1939). Nitrite-containing ice also has the advantage that it preserves both

TABLE V.   The influence of ice containing 0.1 per cent of sodium nitrite on the keeping quality of black cod.

No. of days fish stored in the experimental ices	Count of viable bacteria in poke muscle (colonies per g.)		Results of organoleptic tests		NaNO₂ in p.p.m. of wet muscle in fish stored in ice containing nitrite. Muscle from one side of the fish sampled as in making bacterial counts
	Tapwater ice	Ice containing NaNO₂	Fish from tapwater ice	Fish from ice containing nitrite	
5	300	20	No noticeable difference in external appearance. Odour of poke: Sour	Fresh	88
11	3,260,000	1,120	External appearance: Covered with brownish - yellow slime. Odour of poke: Very sour. Cooked flesh: Slightly stale	Good. Fresh. Edible, apparently fresh	67
13	552,000	51,400	External appearance: Covered with brownish - yellow slime. Odour of poke: Very putrid. Cooked flesh: Slightly stale, flat	Good. Slightly sour. Edible, apparently fresh	350
14	860,000	31,600	Fish in about the same condition in all respects as after 13 days in the experimental ices.		74
16	1,960,000	45,800	External appearance: Similar to that after 13 days. Odour of poke: Very putrid. Cooked flesh: Stale and practically inedible	Fair. Slightly sour. Edible, not unpleasant	...

recently caught fish and fish which have been stored for some days in ordinary ice. This is probably partly due to the fact that sodium nitrite readily penetrates the fish muscle, and that it is undoubtedly a much more effective preservative for fresh and smoked fish than is benzoic acid when used in similar (per cent) concentration in brines in which fillets are immersed (Tarr and Sunderland 1939a, 1939b, 1940).   Ice containing 0.5 per cent of sodium nitrite causes treated fish

to absorb rather large amounts of the salt, and for this reason in most of the experiments 0.1 per cent was employed. The rather great variations in the nitrite content of the muscle of treated fish in a given experiment may be due to some variation in the rate of absorption of the salt in different fish, or to some other unknown cause.

So far, no attempt has been made to overcome the yellow discolouration which arises when the white ventral surface of halibut comes in contact with nitrite-containing ice. No such obvious discolouration has been noticed in either pink salmon or black cod which have been stored in nitrite-containing ice, though freshly clotted blood in the poke region becomes brown due, probably, to the formation of methaemoglobin (Brooks 1937). Mention should be made of the fact that nitrite may in certain instances cause the formation of a very faint pink colour in the muscle of white fish, and increase the intensity of the red colour of the flesh of salmon. The probable cause of this has been referred to elsewhere (Tarr and Sunderland 1939b, and unpub. M.S.). It is intended to pursue this investigation further in the near future.

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The Comparative Value of Preservatives for Fresh Fillets

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ABSTRACT

The effect of a number of chemical preservatives in inhibiting the bacterial "spoilage" of fresh fillets of certain species of Pacific coast fish has been studied. The preservatives were applied by dissolving them in sodium chloride brines in which the fish were immersed. Chloroform (0.7%) markedly improved the keeping quality of treated fish, while hydrogen peroxide (0.1%) had little effect. Sulphurous acid (0.1% SO_2) considerably retarded bacterial "spoilage" but produced an unpleasant flavour. Hydrochloric acid (0.1%) slightly enhanced keeping quality but caused treated fillets to assume an unattractive appearance. Benzoic acid (0.1%) and sodium benzoate (0.1%) were quite effective, the former being slightly more active than the latter. Neither boric acid (0.1%) nor para-hydroxybenzoic acid ethyl ester (0.09%) were as efficient preservatives as benzoic acid or sodium benzoate in similar (per cent) concentration. Both sodium nitrite and potassium nitrite in 0.1% concentration caused a much greater inhibition in bacterial "spoilage" of fresh fillets than did sodium benzoate or benzoic acid, and the probable value of nitrites in fish preservation, as well as their influence on the colour of treated products, is discussed. The efficiency of a given preservative in different cases varied greatly, and in certain experiments a compound which normally exerted a favourable effect on keeping quality was without effect The probable reason for this is discussed.

Most attempts to improve the keeping quality of dressed fish by immersing them in solutions containing bactericidal substances, or by surrounding them with ice in which germicidal compounds have been incorporated, have resulted in failure, or only in a small measure of success, as has previously been pointed out (Tarr and Sunderland 1939a; Tarr and Bailey 1939). The reason for such failures undoubtedly lies in the fact that it is extremely difficult to ensure intimate contact between putrefactive agent and chemical preservative in experiments with dressed fish. One method which appeared to offer considerable possibilities, and which undoubtedly greatly enhances the keeping quality of treated fish, namely that of immersing them in strong salt solutions on board ship during the interval between dressing and icing them (Bedford 1932), has never been adopted. Such treatment so alters their external appearance as to make them unacceptable according to the grading methods in vogue. However, in excised fish muscle (fillets, steaks, etc.) contact between preservative and bacteria is facilitated, and recent work has been directed largely to the study of such products.

148

Since about 1870 numerous publications have appeared regarding the preservation of fish and fish products by chemical means, and only brief reference can be made to them here. At one time boric acid was advocated as a preservative, and was used to prevent reddening of salted fish (Ewart 1886; Stevenson 1899; Tressler 1923; Bronkhorst 1926; Cobb 1927), but more recently benzoates have been employed for these purposes (Cobb 1927; Gibbons 1935). In recent years such varied agents as acids (Metzner 1933b; Gibbons 1934; Notevarp, Hjorth-Hansen and Monssen 1936; Nadeau 1939), carbon dioxide (Killeffer 1930; Coyne 1933; Stansby and Griffiths 1935), ultraviolet light (Tarr, Young and Sunderland 1938; Puncochar, Lanham and Nilson 1939) and hydrogen peroxide (Metzner and Oeser 1938; Metzner, Hutschenreuter and Oeser 1938; Lücke 1938; and Hjorth-Hansen and Karlsen 1939) have been employed to preserve fish.

The literature pertaining to the use of germicidal substances in ices has already been reviewed (Tarr and Bailey 1939), and brief reference has also been made to experiments dealing with the preservation of lightly smoked and fresh fillets (Tarr and Sunderland 1938, 1939a and b). A detailed report of the experiments on the preservation of fresh fillets follows.

METHODS

Bacteria produce changes in fish which are more or less objectionable to those who prefer really fresh fish, and such changes may be called "spoilage."

No single and entirely satisfactory chemical, physical or biological test by which the degree of bacterial "spoilage" of all kinds of sea fish can be accurately and quantitatively measured, has yet been found. For the purpose of the experiments to be described viable bacterial counts and organoleptic tests were arbitrarily selected as the best available criteria of bacterial "spoilage". The "trimethylamine test," which has apparently given quite satisfactory results under certain conditions, was used only in one experiment, since benzoic acid, benzoates and para-hydroxybenzoic acid ethyl ester markedly suppress trimethylamine formation in "spoiling" fish muscle without effecting a corresponding inhibition in bacterial multiplication, as this and previous work (Tarr and Sunderland 1938; Tarr and Bailey 1939) has shown. Also, only certain of the organisms causing fish "spoilage" form trimethylamine (Watson 1939; Tarr 1938, 1939).

Viable bacterial counts and trimethylamine determinations were made by methods previously described (Tarr and Bailey 1939). The amount of sodium nitrite in treated fish was determined as follows. Aqueous extracts of the muscle were prepared following the technique used in making bacterial counts, the protein was precipitated with mercuric chloride solution, and the amount of nitrite present in the filtrate, or in suitable dilutions prepared from it, was determined colorimetrically, employing the Greiss-Ilsovay reagent in the method outlined by the Association of Official Agricultural Chemists (1925).

Organoleptic tests were made by the writers, though, whenever possible, the assistance of others was obtained. These tests included examination of the uncooked fish for odour, and of the fish baked for 10 minutes at about 300°C.

in an electric oven for both odour and flavour. For purposes of brevity the results of organoleptic tests have been recorded as in table I. Organoleptic tests have been made only in experiments where it was considered that they would give information of value.

In the first few experiments small fillets which were cut to approximately the same length, width and thickness were used. Later this technique was altered, pieces of fish muscle cut to standard size being treated, thereby ensuring a more uniform penetration of preservative or sodium chloride. In all experiments the skin was removed from the fillets. Tests were at first carried out by dipping the fillets in 2 per cent sodium chloride brines at a temperature of approximately 20°C., with or without added preservative. This technique was subsequently altered when it became evident that a brief immersion of fillets in strong sodium chloride brines was itself a strong deterrent to bacterial putrefaction, and that, for reasons to be given later, brines kept at temperatures near the freezing point of water were preferable in certain respects to those used at higher temperatures. After the brine treatment the fillets were usually placed in sterilized 500- or 750-ml. covered glass beakers or 20-cm. petri dishes, though in certain experiments they were wrapped in cellophane paper. The fish was usually stored at 1.5°C., though occasionally a higher temperature was employed. The liquid which accumulated was drained from the containers daily. In most experiments the samples were stored until the untreated fish became stale, when all fillets were examined. Lower bacterial counts and better odour and flavour in treated than in untreated samples were taken to indicate an improvement in keeping quality. In other experiments a comparison of the length of time required for treated and untreated fillets to arrive at approximately the same stage of staleness, as indicated by viable bacterial counts and odour, was used as a criterion of improvement in keeping quality.

Usually it has been possible to give only a very approximate estimate of the number of days which elapsed between the catching of the fish and the time at which they were used for experimental purposes. Thus the expression, "five days old in ice" signifies that the fish used were stored for about five days in ice during the time which elapsed between capture (dressing and washing) and experimental use.

<div align="center">EXPERIMENTAL</div>

A. FILLETS TREATED IN WEAK (2%) SODIUM CHLORIDE BRINES

EXPERIMENT 1.

One-litre portions each of five solutions having the composition shown in table I were prepared. Small flounders about 1 day old in ice were filleted and 200 ± 10 g. of the fillets were immersed in each of these solutions for 1 hour at about 20°C. with occasional stirring. After 3 days' storage at 1.5°C., 3 fillets were removed from each container, and, after mincing them together with sterile scissors in the usual manner, viable bacterial counts and determinations of the trimethylamine content of the fish were made, with the results given in table I The remaining fillets were re-incubated at 22°C. for 17 hours in order to accelerate decomposition, after which further examinations were made (table I).

TABLE I. Experiment 1. Spoilage (as judged by numbers of viable bacteria, trimethylamine content and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Stored 3 days at 1.5°C.		Stored 3 days at 1.5°C. +17 hr. at 22°C.		Order of preference of treatment according to organoleptic tests**	
	Bacterial counts*	Mg. tri-methylamine nitrogen per 100 g.	Bacterial counts*	Mg. tri-methylamine nitrogen per 100 g.	Fish uncooked (3 judges)	Fish cooked (2 judges)
1. None.............	20,200	0.17	26.4 ×10⁶	24.2	4, 4, 3	2, 3
2. 0.1% benzoic acid..	4,760	0.18	0.8 ×10⁶	0.32	1, 1, 1	1, 1
3. 0.09%para-hydroxy-benzoic acid ethyl ester...	4,920	0.14	8.7 ×10⁶	0.37	1, 1, 1	1, 1
4. 0.1% sulphur di-oxide..........	5,800	1.03	0.25×10⁶	13.2	5, 5, 5	3, 5
5. 0.1% hydrogen per-oxide	9,920	0.13	11.5 ×10⁶	10.6	3, 3, 4	2, 4

*In this and in subsequent tables bacterial counts are given as numbers of colonies per g. of wet fish muscle, the figures representing averages of duplicate determinations

**In cases where there was equivalence of preferences the samples in question were given the same number. Thus in the above table the order of preference according to cooked flavour was in one case (2, 3) (1, 5), 4 (brackets indicating equivalence of preference). This was therefore recorded as first place for samples 2 and 3; second place for samples 1 and 5 and third place for sample 4.

It is evident that all of the four preservatives studied retarded to a greater or lesser degree the bacterial "spoilage" of flounder muscle. The effect of the preservatives was more marked after the longer storage period in the case of the benzoic acid and sulphurous acid treatments, as judged by bacterial counts; but this is not true for hydrogen peroxide, a result which might be expected since this compound has a purely transient effect, being rapidly decomposed, presumably by the catalase present in the muscle. Both benzoic acid and para-hydroxybenzoic acid ethyl ester gave considerable protective action, though the former was more effective than the latter. Both almost entirely suppressed trimethylamine formation in the treated muscle without effecting nearly such a marked inhibition in the increase in viable bacteria. Sulphur dioxide had a strong bactericidal action, but produced such an unpleasant rancid odour and flavour in the treated fish, presumably due to its reaction with the oils present (Denstedt and Brocklesby 1935) that its use was not considered further.

Sulphur dioxide apparently effects a chemical reduction of trimethylamine oxide to trimethylamine, for the increase in the viable bacterial population is hardly great enough to account for the relatively large amount of trimethylamine in the treated muscle.

EXPERIMENT 2

The relative effectiveness of benzoic acid and sodium benzoate as preservatives for fresh fillets was determined, the technique being similar to that followed in experiment 1. Small flounders 3 days old in ice were filleted and 12 fillets (150 to 160 g. of fish) were immersed for 1 hour at about 20°C. in 1-litre portions of each of 5 brines (table II). The treated fillets were stored at 1.5°C. for 10 days. Viable bacterial counts were then made after mincing together 3 fillets selected at random from each of the 5 lots, and also organoleptic tests on the remaining fillets (table II). Both bacterial counts and organoleptic tests indicate a marked superiority in keeping quality of the treated over the untreated fillets. The viable bacterial counts showed that 0.1% benzoic acid was only slightly superior as a preservative to 0.1% sodium benzoate, the difference obtained being hardly significant. This result is probably explained by the fact that, although the free acid is a much more effective germicide than the benzoate (Goshorn, Degering and Tetrault 1938), it is possibly neutralized as soon as it comes in contact with the fish muscle. It will be noticed that the organoleptic tests were not sufficiently sensitive to indicate significant differences among the fillets treated with preservatives.

TABLE II. Experiment 2. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Bacterial counts	Order of preference of treatment according to organoleptic tests tests (3 judges)	
		Fish uncooked	Fish cooked
1. None	1398×10^6	5, 5, 2	5, 5, 3
2. 0.1% benzoic acid	46.6×10^6	1, 1, 4	1, 1, 3
3. 0.1% sodium benzoate	144.8×10^6	2, 3, 4	2, 4, 4
4. 0.5% sodium benzoate	3.3×10^6	2, 2, 2	2, 2, 2
5. 1.0% sodium benzoate	0.19×10^6	1, 2, 3	1, 3, 2

EXPERIMENT 3

A halibut (*Hippoglossus stenolepis*) weighing about 6 lb. (3 kg.) which had been air frozen when still fresh, wrapped in cellophane and stored 5 months at −20°C., was thawed by placing in a 20°C. incubator for 16 hours. Pieces of muscle approximately $7.5 \times 7.5 \times 2.0$ cm. were cut from this fish, and 4 of these brined for 1 hour at approximately 20°C. in 1-litre portions of each of the 4 solutions, the composition of which is given in table III. After 9 days' storage at 1.5°C., 2 pieces of muscle from each lot were minced together for bacterial counts, the remaining 2 being used for organoleptic tests (table III).

In this experiment it will be observed that the differences obtained between treated and untreated fish were insignificant as far as bacterial counts were concerned, while the results of organoleptic tests were not very conclusive in that

Table III. Experiment 3. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of halibut fillets in 2% sodium chloride brine after treating with the preservatives listed.

Preservative	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
		Fish uncooked	Fish cooked
1. None..............................	1146×10^6	3, 4, 4	2, 3, 4
2. 0.1% benzoic acid...................	1034×10^6	1, 2, 3	2, 2, 2
3. 0.09% para-hydroxybenzoic acid ethyl ester................................	812×10^6	1, 1, 1	1, 1, 1
4. 0.1% sodium benzoate................	1610×10^6	2, 2, 3	3, 3, 4

they did not in all cases indicate preference for the treated samples. The probable reason for this apparent discrepancy is discussed on page 160.

EXPERIMENT 4

Small fillets were cut from flounders which were less than 1 day old in ice, and 6 were immersed for 1 hour at about 20°C. in 1-litre portions of each of 6 brines, the composition of which is given in table IV. The fillets were stored at

TABLE IV. Experiment 4. "Spoilage" (as judged by numbers of viable bacteria and organoleptic tests) of flounder fillets after treating in 2% sodium chloride brines with the preservatives listed.

Preservatives	Wt. of fish added (g.)	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
			Fish uncooked	Fish cooked
1. None...................	167	80.0×10^6	3, 5, 5	2, 3, 4
2. 0.7% chloroform (approx.)	165	0.26×10^6	1, 2, 3	2, 2, 3
3. 0.1% potassium nitrite....	180	0.48×10^6	1, 4, 1	1, 2, 5
4. 0.1% boric acid..........	180	8.2×10^6	1, 2, 3	1, 2, 6
5. 0.073% hydrochloric acid.	170	1.5×10^6	2, 5, 5	1, 1, 3
6. 0.1% benzoic acid........	181	14.4×10^6	1, 1, 4	2, 2, 3

1.5°C. for 10 days in the usual manner, and at the end of this time 2 from each lot were subjected to bacteriological analysis, the remainder being used for organoleptic tests (table IV). The results of the bacteriological tests show that both potassium nitrite and chloroform treatment caused an extremely marked improvement in keeping quality. Hydrochloric acid, boric acid and benzoic acid, while definitely retarding bacterial "spoilage," did not produce such a noticeable improvement as the first named compounds. Organoleptic tests did not indicate

such marked differences as the viable bacterial counts, especially when the cooked fish was employed as criterion. 'It appears that bacteriological decomposition had not proceeded far enough to cause obvious differences in the flavour of the cooked samples; there is no doubt that many of the volatile compounds which aid in detecting differences by smell alone are driven off during the cooking process.

EXPERIMENT 5

An air-frozen halibut weighing about 10 lb. (4.5 kg.), which had been frozen when fresh and stored for 5 to 6 months at −20°C., was thawed at 22°C. Sixteen pieces of the muscle about 7.5×5×1.5 to 2.0 cm. thick were cut, and 4 of these treated for 1 hour at approximately 20°C. in 1-litre portions of each of the 4 solutions, the composition of which is given in table V. After storing 12 days at 1.5°C., 2 fillets from each lot were used for determination of numbers

TABLE V. Experiment 5. "Spoilage" (as judged by numbers cf viable bacteria and organoleptic tests) of halibut fillets after treating with various concentrations of potassium nitrite in 2% sodium chloride brine.

Percentage of potassium nitrite	Wt. of fish added (g.)	Bacterial counts	Order of preference of treatment according to organoleptic tests (3 judges)	
			Fish uncooked	Fish cooked
1. None	250	964 ×10⁶	2, 4, 4	4, 4, 4
2. 0.02%	274	158 ×10⁶	1, 2, 3	3, 3, 3
3. 0.05%	263	20.6 ×10⁶	1, 1, 3	1, 2, 2
4. 0.10%	254	0.66×10⁶	1 ,1, 2	1, 1, 2

of viable bacteria, and 2 for organoleptic tests (table V). The results show that potassium nitrite treatment very markedly improves the keeping quality of halibut fillets both as regards bacterial counts and organoleptic tests.

At this stage it was realized that more uniform results as regards penetration of preservative and salt could be expected by using pieces of fish muscle cut to a definite size, and this was done for all subsequent experiments.

EXPERIMENT 6

Red cod (genus *Sebastodes*) about 2 days old in ice were filleted and 36 pieces of muscle 5×5×2 cm. were cut from them. Nine different brines in quantities of 1 litre were prepared (table VI), and 4 pieces of red cod were immersed in each of them for 1 hour at about 20°C. After 12 days' storage at 1.5°C. the fillets were examined in the usual manner. In this experiment viable bacterial counts (table VI) showed no significant differences in keeping quality of the treated fillets, with the single exception of those exposed to chloroform, as compared with those not treated with preservatives. Organoleptic tests indi-

TABLE VI. Experiment 6. "Spoilage" (as judged by viable bacterial counts) of red cod fillets after treating in 2% sodium chloride brine with the preservatives listed.

Preservative	Bacterial counts
None...	296×10^6
0.05% sodium nitrite....	270×10^6
0.05% potassium nitrite........................	284×10^6
0.10% sodium benzoate.	1686×10^6
0.10% benzoic acid.................	1360×10^6
0.10% hydrogen peroxide..................	1104×10^6
0.09% para-hydroxybenzoic acid ethyl ester......	1152×10^6
0.7% chloroform	0.114×10^6
0.1% boric acid..........	342×10^6

cated that only in the case of the chloroform treated fish was there any noticeable improvement in the condition of the fillets. Almost identical results were obtained when using halibut fillets in another similar experiment with the same preservatives.

EXPERIMENT 7

Sixteen pieces of muscle $5 \times 5 \times 2$ cm. were cut from a halibut about 7 days old in ice. One-litre quantities of 4 brines, the composition of which is recorded in table VII, were prepared, and 4 pieces of the fish were immersed in each

TABLE VII. Experiment 7. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of halibut fillets after treating with various concentrations of this preservative in 2% sodium chloride brine.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet halibut muscle after storing at at 1.5°C. for:		Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
	3 days	6 days		Fish uncooked	Fish cooked
1. None	Not appreciable	Not appreciable	$1,906 \times 10^6$	4, 4	4, 4
2. 0.02%	42	9	$1,626 \times 10^6$	3, 3	3, 3
3. 0.05%	124	27	852×10^6	2, 2	2, 2
4. 0.10%	184	72	4.5×10^6	1, 1	1, 1

of these for 1 hour at a temperature of about 20°C. The amount of sodium nitrite present in the treated muscle was determined after 3 and 6 days' storage at 1.5°C., 1 fillet being used for the determination after the 3-day period (table VII). The number of viable bacteria in 1 fillet from each lot was determined after 6 days at 1.5°C., organoleptic tests being made on the remaining fillets (table VII). The results show that sodium nitrite, in concentrations lower than 200 parts per million (p.p.m.), strongly retarded bacteriological "spoilage" in the treated fillets. It will also be observed that the amount of nitrite in the

treated fish decreases on storage, presumably due to its reduction to ammonia by certain of the bacteria present.

EXPERIMENT 8

Four pieces of halibut muscle 5×5×2.5 cm. cut from a fish 5 days old in ice were immersed for 5 hours in 1-litre portions of 2 different brines (table VIII) which were maintained at 7 to 8°C. Sodium nitrite was determined in one fillet from each brine immediately subsequent to brining, and again after 9 days' storage of the remaining fish at 1.5°C., when bacteriological counts and organo-

TABLE VIII. Experiment 8. "Spoilage" (as judged by viable bacterial counts) and sodium nitrite content of halibut fillets treated in 2% sodium chloride brine with and without 0.05% of sodium nitrite.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet halibut muscle		Bacterial counts
	Immediately after treatment	After 9 days' storage at 1.5°	
None	Not appreciable	Not appreciable	166×10⁶
0.05%	211	216	0.4×10⁶

leptic tests were also made (table VIII). In this experiment a very marked improvement in keeping quality was obtained by the sodium nitrite treatment, as the viable bacterial counts show. Organoleptic tests by two independent judges showed that the treated fillets were much superior to the untreated ones. In this experiment the viable bacterial content of the nitrite-treated fish was apparently not high enough to cause a reduction of the nitrite.

EXPERIMENT 9

A white spring salmon (*Oncorhynchus tschawytscha*) about 1 day old in ice was filleted, and 16 pieces of muscle 5×5×2 cm. were cut from the fillets. One-

TABLE IX. Experiment 9. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of white spring salmon fillets treated with various concentrations of sodium nitrite in 2% sodium chloride brine.

Percentage of sodium nitrite	Sodium nitrite in p.p.m. of wet muscle after 9 days' storage at 1.5°C.	Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
			Fish uncooked	Fish cooked
1. None	Not appreciable	2560×10⁶	4, 4	4, 4
2. 0.05%	153	125×10⁶	3, 3	3, 3
3. 0.10%	400	2.2×10⁶	1, 1	2, 2
4. 0.20%	780	0.66×10⁶	1, 1	1, 1

litre portions of 4 different brines (table IX) were prepared, and 4 of the experimental fillets were immersed for 1 hour at 20°C. in each of them. After 9 days' storage at 1.5° the fillets were examined for numbers of viable bacteria and the amount of sodium nitrite, organoleptic tests also being made (table IX). The results show that sodium nitrite treatment strongly delayed bacterial "spoilage" in the treated fillets, especially in the higher concentrations.

EXPERIMENT 10

Twenty pieces of halibut muscle 5×5×2 cm. were cut from a fish 4 days old in ice. Four pieces were immersed for 1 hour at approximately 20°C. in 1-litre portions of 5 solutions, the composition of which is given in table X.

TABLE X. Experiment 10. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of halibut fillets after treating in 2% sodium chloride brine with the preservatives listed

Preservative	Sodium nitrite in p.p.m. of wet halibut muscle		Bacterial counts	Order of preference of treatment according to organoleptic tests (4 judges)	
	Immediately after treatment	After 11 days at 1.5°C.		Fish uncooked	Fish cooked
1. None..	Not appreciable	Not appreciable	854×10^6	3, 3	5, 5, 5, 5
2. 0.05% sodium nitrite	163	176	66.8×10^6	2, 2	3, 3, 3, 3
3. 0.10% sodium nitrite	364	372	0.07×10^6	1, 1	1, 1, 2, 2
4. 0.20% sodium nitrite	600	710	0.019×10^6	1, 1	1, 1, 2, 2
5. 0.20% sodium benzoate	...	. .	2760×10^6	3, 3	4, 4, 4, 4

One fillet from each brine was analysed for sodium nitrite content immediately after treatment. After 11 days' storage at 1.5°C. one of the remaining fillets was used in each case to determine the numbers of viable bacteria present, organoleptic tests being made on the remainder (table X). In this experiment sodium nitrite exerted a decidedly favourable effect on the keeping quality of the fish, while sodium benzoate was quite inactive in this respect, the bacterial count in this case being actually slightly higher than that obtained in the control.

B. FILLETS TREATED IN STRONG (15 OR 20%) COLD SODIUM CHLORIDE BRINES

In view of the facts that a brief immersion of fillets in fairly strong (15 to 20%) cold (0 to 5°C.) sodium chloride brines has the advantage of imparting a "sheen" or "gloss" to their surfaces which distinctly improves their external appearance, and that it also causes the fish to absorb sufficient salt to make it more palatable on cooking, the influence of such treatment on keeping quality, with and without added preservatives, was investigated. Experiments by one of us (P. A. S.), in connection with the mild curing of salmon, have verified the findings of Scofield (1925) that, when the "sliming" of the sides is carried

out in brine held at 2 to 5°C. the surface of the fish becomes "case hardened" resulting in an enhancement in the appearance of the cured fish. There is some indication that both sodium chloride and sodium nitrite penetrate more slowly in fish treated in cold brines, but so far the experimental evidence accumulated has been far too meagre to warrant drawing definite conclusions regarding the mechanism of this so-called "case hardening" effect and its influence on salt penetration. Experiments by Cooper and Linton (1936) have shown that treatment of fillets intended for smoking in cold brines results in a better surface sheen than when warmer brines are used.

EXPERIMENT 11

Ling cod (*Ophiodon elongatus*) about 3 days old in ice were obtained, and a number of pieces of muscle 5×5×2 cm. cut from them. Five pieces were immersed in 3-litre lots of each of two brines (table XI) for 5 minutes at 2°C., the

TABLE XI. Experiment 11. "Spoilage" (as judged by viable bacterial counts and organoleptic tests) and sodium nitrite content of ling cod fillets after different treatments.

Treatment	Sodium nitrite in p.p.m. of wet muscle immediately after treatment	Bacterial counts	Order of preference of treatment according to organoleptic tests (2 judges)	
			Fish uncooked	Fish cooked
Unbrined fillets	. . .	170 ×10⁶	3, 3, 3	3, 3, 3
20% sodium chloride brine	. . .	33.8 ×10⁶	2, 2, 2	2, 2, 2
20% sodium chloride brine + 0.25% sodium nitrite	269	1.18×10⁶	1, 1, 1	1, 1, 1

remaining 4 being retained untreated as controls. After treatment the brined fillets were permitted to drain for 15 minutes in a 1.5°C. room, nitrite was determined in 1 of the treated fillets, and then all 3 lots were wrapped separately in moisture-proof cellophane and stored at 10°C. for 3 days. Bacterial counts and organoleptic tests (table XI) were then made, using 2 fillets for each of these determinations. The results show that a brief immersion of the fillets in cold 20% sodium chloride brine materially improved their keeping quality, and that the addition of sodium nitrite to the brine even further reduced the rate of bacterial "spoilage".

EXPERIMENT 12

Pieces of muscle 5×5×2 cm. were cut from red spring salmon (*Oncorhynchus tschawytscha*) 3 days old in ice, and from halibut 6 to 7 days old in ice. In the case of each fish 4 fillets were retained as untreated controls, and 5 were brined for 5 minutes at 5 to 6°C. in each of 2-litre portions of the solutions, the composition of which is given in table XII. After treatment the fillets were drained for 30 minutes at a temperature of about 1.5°C., one fillet from each lot being examined for sodium nitrite content (table XII) and they were then wrapped

in lots of 4 in moisture-proof cellophane and stored at 1.5°. They were examined regularly, and when they exhibited a definite "stale" odour bacterial counts were made, with the results given in table XII. It is important to note that this method of attempting to indicate a given degree of "spoilage" by the simple organoleptic test is necessarily very empirical, but it is rather doubtful if any other method would have yielded more accurate results. The criticism might be offered that it would have been more accurate to store the fillets until they had a closely similar bacterial count rather than to use this merely as a supporting test. For obvious reasons such a method presents great experimental-difficulties, and it is extremely doubtful if the results obtained would have been much more valuable. Experience has shown that bacterial counts usually must vary at least ten times before very significant differences in "spoilage" are observed, as

TABLE XII. Experiment 12. Composition of brines, length of time fillets were stored at 1.5°C. before they became "stale," and nitrite and bacterial contents of fillets.

Treat-ment no.	Composition of the brine used and length of time the fillets were immersed	No. of days fillets stored at 1.5° before they became "stale"		Sodium nitrite in p.p.m. of wet muscle immediately after the fillets were brined		Bacterial count at the end of holding period indicated	
		Halibut	Salmon	Halibut	Salmon	Halibut	Salmon
1	Controls (unbrined).	10	14	Not appreci-able	Not appreci-able	1100×10^6	506×10^6
2	15% sodium chloride— 5 min.	15	16	"	"	136×10^6	228×10^6
3	10 min.	19	21	"	"	122×10^6	236×10^6
4	+0.1% sodium nitrite, 5 min.	24	29	85	49	1522×10^6	178×10^6
5	+0.1% " " 10 min.	29	32	110	50	294×10^6	282×10^6
6	+0.2% " " 5 min.	30	33	160	61	342×10^6	624×10^6
7	+0.2% " " 10 min.	32	34	169	67	62.8×10^6	126×10^6

the experimental results in this case indicate. The combination of organoleptic test and viable bacterial count was therefore arbitrarily selected as indicating approximately the same degree of "spoilage." It will be seen from the figures given that in the case of both salmon and halibut fillets the brining treatment alone greatly increased the length of time at which they could be stored at 1.5°C. before they became "stale" and had a high bacterial content, and that the addition of sodium nitrite to the brines used caused an even more marked increase in keeping quality. It is important to note that, for a given concentration of $NaNO_2$ in the brine, very little more was apparently absorbed in 10 minutes than in 5 minutes. The salmon fillets, as might be expected from the fact that the flesh is very oily, took up much less sodium nitrite than did the halibut fillets.

DISCUSSION

The purpose of the experiments described has been to discover some method of treating fresh fillets which would improve their keeping quality, not occasion an unpleasant appearance nor in any way damage their flavour, and at the same time comply with the pure food regulations (Food and Drugs Act, Ottawa, 1938). Preliminary work was concerned with studying the effect of a number of preservatives, most of which had been previously used in fish or fish products. Many of these substances are not permitted by law in meat or fish products in this country, but they were studied partly because certain countries do permit their use, and partly because some standard of comparison was required.

It will be noticed that there was little uniformity in the bactericidal action of a given preservative in different experiments: in certain cases the effect was very pronounced, in others only moderate, while in some cases there was no observable activity. The reason for this is not clear, but it may be due to the fact that in some cases of fish spoilage the predominating organisms are more sensitive to a given germicide than in others. Thus in experiment 10 sodium benzoate was without noticeable preservative action, while in experiment 2 it was extremely effective.

That saltpetre (potassium nitrate) has been used in the curing of meats for many centuries is certain, but the exact time and circumstances surrounding its introduction are obscure. Kerr, Marsh, Schroeder and Boyer 1926; Horovitz-Vlasova 1931; Reiss, Meyer and Müller 1928; Jones 1933 and Khristodulo 1938 have all summarized the literature regarding the use of this compound in meats. From their discussions it appears that early in the present century it was made known that certain of the bacteria present in the pickles used for meat curing reduced nitrates (saltpetre) to nitrites, and that this compound in turn decomposed, yielding traces of nitric oxide which reacted with the haemoglobin of the meat to form nitrosohaemoglobin. It is this last named compound which is apparently responsible for the bright red colour characteristic of many types of cured meats (Brooks 1937). As far as can be ascertained from the literature, little or nothing is known regarding the possible bacteriostatic (or bactericidal) action of nitrites in meat pickles or in cured meats themselves. It has, however, long been known that nitrites inhibit certain of the dehydrogenase enzymes of bacteria and in concentrations of the order of 0.4 per cent entirely prevent growth of certain organisms (Stephenson 1939). One of us (H.T.) has found that sodium nitrite has a marked bacteriostatic, and, in certain instances, a bactericidal action on certain of the organisms involved in fish spoilage. This action is being studied in detail.

As far as can be ascertained from a survey of the available literature, nitrites have not been employed previously in the preservation of fresh or smoked fish, or in the treatment of fish products. In 1899, Tower (cited by Griffiths 1937) found that washing fish in 10 per cent solutions of potassium nitrate did not improve their keeping quality. Taylor (1923) stated that potassium nitrate was little used in fish curing because of the red colour it caused, and because hydrogen sulphide formation was not troublesome. He apparently believed that the

nitrates in cured meats oxidized hydrogen sulphide to water and sulphur dioxide which acted as a sterilizing and bleaching agent.

It is not yet clear why organisms associated with "spoilage" of fish muscle are so frequently sensitive to relatively small concentrations of nitrite. Tanner and Evans (1934) have pointed out that the ordinary bacterial flora of meat is inhibited by sodium nitrite in concentrations as low as 300 parts per million in pickles, while certain pure cultures of putrefactive anaerobes required very much higher concentrations to cause significant inhibition. Probably such factors as type of organism, environment (especially as regards aerobic or anaerobic conditions), ability to develop nitrite-tolerance, etc., all play some part in determining the sensitivity of bacteria to nitrites.

That nitrites are extremely effective in inhibiting bacterial multiplication in and on the surfaces of recently caught dressed fish has been shown by the writers in their experiments with ices containing sodium nitrite (Tarr and Sunderland 1939c). Nitrite ice has been found to be a very much more effective preservative than benzoic acid ice (Tarr and Bailey 1939) for dressed fish.

It has also been found that nitrites have, in many instances, a noticeable effect on the colour of treated fish muscle. Thus in certain species of salmon a more or less marked intensification of the normal red or pink colour results from nitrite treatment, the effect being apparently more noticeable in some species than in others, and seeming to vary somewhat in individual fish. In salmon this intensification of colour, though sometimes noticeable in raw fish, is more marked subsequent to heating the muscle, as in cooking, canning or smoking. Hayasi (1933) showed that haemoglobin (myoglobin) is present in the muscle of many of the species of fish which he examined spectroscopically, and it would seem highly probable that the intensification of colour effected by nitrite treatment is due to the formation of nitrosohaemoglobin, as is the case with meats. This point requires investigation. In most "white" fish nitrite treatment causes no apparent change in colour or only a faint pinkness in the muscle. Whether this incidental intensification of colour is desirable from a commercial standpoint is an open question.

Preliminary experiments made in attempts to ascertain what effect changes in pH and salt concentration of the brines may have on the efficiency of a given germicide have so far yielded inconclusive results. In the first place, although the pH of a brine may readily be made acid or alkaline, the pH of fish treated with such brines tends to remain from about 6.4 to 7.0 unless a great excess of acid or alkali is added. Undoubtedly changes in pH can be effected by adding very large amounts of acids or alkalies to a brine, but such treatment has a rather detrimental effect on the appearance of treated fillets. The study of the effect of sodium chloride concentration upon the germicidal efficiency of a given preservative is likewise complicated. The problem in this case then resolves itself into a study of the effect of the preservative in question on organisms which are and are not inhibited by sodium chloride treatment.

In closing it is necessary to call attention to the regulations governing the use of preservatives in fish and fish products. In Canada benzoates are not allowed, but sodium nitrite in concentrations not exceeding 200 parts per million

(0.02%) is permitted by law in cured meats (including fish) (Food and Drugs Act, Ottawa, 1938). Certain other countries have less stringent regulations; thus in Germany (Metzner 1933a) benzoates and a large number of preservatives not permitted in Canada can be used. Though nitrites in large quantities are undoubtedly toxic to man (Reiss et al. 1928; Behre 1939), it would appear that adults can ingest at least 200 mg. of sodium nitrite daily without ill effects: this amount would be present in 1 kg. of fish containing 200 parts per million of sodium nitrite. Also nitrites are consumed regularly in various types of cured meats without apparent injury to health.

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Specificity of Triamineoxidease

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ABSTRACT

The substrate-specificity of a newly described enzyme common to six bacterial species, comprising five different genera, isolated from such widely divergent sources as decomposing fish, well-water and surface taint butter, was investigated. Of the various substrates studied only trialkylamine oxides having the general structure $R_3 \equiv N = 0$ were activated with subsequent reduction, the corresponding volatile base being formed in each case. Betaine, choline, acetylcholine, ergothioneine and stachydrine containing an atomic group similar to the above were not activated. The designation "triamineoxide*ase*" is proposed for this enzyme.

In previous papers the writer showed that certain bacterial cells possess an enzyme which activates trimethylamine oxide rendering it capable of reduction by many dehydrogenase systems of lower potential level. Working independently, Watson (1939, a and b) came to a similar conclusion, and stated that, "From these results it is evident that trimethylamine oxide, like nitrate, requires a degree of activation by the bacterial cell." The writer (1939, a and b) suggested that the activating principle be named "Trimethylamineoxide*ase*". In this paper reasons are given for substituting the more general and concise designation "Triamineoxide*ase*" (Tarr 1940) for the enzyme. In previous work (Tarr 1939b) an attempt to obtain a cell-free preparation of the enzyme by an autolytic method was unsuccessful, and therefore in the experiments to be described intact bacterial cells have been employed. It is possible that a cell-free preparation of the enzyme could be obtained using a wet crushing mill of the type described by Booth and Green (1938).

EXPERIMENTAL

CULTURES

The following organisms employed in previous experiments (Tarr 1939b) were used: Cultures nos. 1 (*Micrococcus*), 18 (*Micrococcus*) and 22 (*Achromobacter*). Cultures C2, C8 and 4 were obtained from Professor Eagles of the University of British Columbia. They possessed the following characteristics: Culture C2, isolated from butter with "surface taint" and capable of producing this defect experimentally; member of the genus *Escherichia*. Culture C8, isolated from a similar source and capable of producing the same defect in butter as culture C2; it appears to be a member of the genus *Aerobacter*. Culture 4, isol-

187

ated from well-water obtained from a creamery noted for its production of butter with surface taint, and capable of producing this defect under experimental conditions; it appears to be a member of the genus *Pseudomonas*.

Washed suspensions of the cells of these organisms were prepared as previously described (Tarr 1939b) using 0.85 per cent sterile sodium chloride solution, adjusted to approximately pH 7.0 with phosphate buffer, as washing and suspending medium.

ORGANIC BASES

Trimethylamine oxide. Prepared by oxidizing trimethylamine (Eastman Kodak) with hydrogen peroxide, and crystallizing the resulting di-hydrate of trimethylamine oxide $(CH_3)_3N=O.2H_2O$, from ethyl alcohol (Dunstan and Goulding 1899, a and b). The melting point, 96°C., agreed with that of the compound as described. The oxide was further identified by means of its picrate $(CH_3)_3N=O.C_6H_2(NO_2)_3OH$, for which Dunstan and Goulding (1899b) reported as the melting point 196 to 198°C. The present preparation melted at 195 to 196°C.

Triethylamine oxide. Prepared by oxidizing triethylamine (Eastman Kodak, B.P. 88-90°) with hydrogen peroxide (Dunstan and Goulding 1899b). The oxide thus obtained is a very deliquescent crystalline compound, and when exposed to air under normal laboratory conditions exists as a thick syrup containing about 80 per cent of triethylamine oxide as estimated by reducing its aqueous solutions with Devarda's alloy and hydrochloric acid (Lintzel 1934). Whether or not this oxide contains water of crystallization is apparently not known. This compound was identified by means of its picrate $(C_2H_5)_3N=O.C_6H_2(NO_2)_3OH$, with the melting point 164 to 166°C., agreeing with that of Dunstan and Goulding (1899b). (*Note:* On recrystallization the picrate melted at 173°C.).

Tri-n-propylamine oxide. Prepared by oxidizing tri-n-propylamine (Eastman Kodak, B.P. 155-157°) with hydrogen peroxide in ethyl alcohol solution at 60°C. (Dunstan and Goulding 1899b). This compound, like the ethyl oxide, is very deliquescent, and it is apparently not known whether it contains any water of crystallization. Tri-n-propylamine oxide was identified by means of its picrate $(C_3H_7)_3N=O.C_6H_2(NO_2)_3OH$, M. P. 129°C., agreeing with that of Dunstan and Goulding (1899b).

Ergothioneine. Prepared by Prof. Eagles of the University of British Columbia.

Stachydrine. Prepared by Dr. H. B. Vickery, Connecticut Agricultural Experiment Station.

Choline hydrochloride, Betaine hydrochloride and Acetylcholine bromide were Eastman Kodak products.

These compounds were dissolved in distilled water, neutralized, where necessary, with sodium hydroxide solution, and sterilized by means of a Seitz filter. The solutions were prepared shortly before use in 0.025, 0.05 or 0.10 M concentration, and were stored at approximately 1.5°C. In the case of the highly deliquescent triethylamine oxide and tri-n-propylamine oxide the strength of the solutions prepared was checked by reducing the oxide by Lintzel's method (1934),

the amount of volatile base formed then being determined. Standard solutions of the other substances used were prepared by weighing the crystalline compounds.

VOLATILE BASE FORMATION

As in previous experiments (Tarr 1939b) volatile base formation from the various compounds employed was studied only under anaerobic conditions, using Thunberg tubes and relatively aseptic conditions in order to avoid serious external contamination. Each tube received 1 ml. of an 0.025, 0.05 or 0.10 M solution of the organic base; 1 ml. of a solution containing equal parts of 0.1 M glucose and 0.1 M sodium lactate (mixed oxidizable substrate); 1 ml. of 0.2 M phosphate buffer pH 7.0 and 2 ml. of bacterial suspension. The tubes containing the solutions were evacuated thoroughly at a water pump, and were then incubated at 25°C. for about 18 hours. At the end of this time the whole contents of each tube were washed into a laboratory-made "Conway dish" and, after adding 0.5 ml. of strong formaldehyde and 1 ml. of a saturated aqueous solution of potassium carbonate, the amount of volatile base was determined in the usual manner. All experiments were made in duplicate, the results being given in table I.

It will be seen from table I that all six cultures studied exhibited a similar specificity toward each of the organic bases investigated. Trimethylamine oxide and triethylamine oxide were strongly activated and, with the single exception of the action of culture 4 on the ethyl compound, about 80 to 100 per cent of the theoretical amount of volatile base was formed under the experimental conditions. Tri-n-propylamine oxide was also activated, but much less strongly than its methyl and ethyl analogues, only from 2.4 to 17.2 per cent of the oxide being reduced under the experimental conditions. It will be observed that heating the bacteria for 10 minutes at 80°C. destroyed their power of reducing the three oxides. From betaine, choline, acetylcholine and ergothioneine, all of which possess a

$$(CH_3)_3 \equiv N \underset{\diagdown C \equiv}{\overset{\diagup O -}{}} $$ grouping, the amount of volatile base formed was, with the single

exception of the last-named compound, practically insignificant, and was approximately identical in the case of both unheated and heat-inactivated organisms. It will be seen that a small amount of volatile base (about 7 per cent of the theoretical amount calculated as $(CH_3)_3N$) was recovered from ergothioneine in the case of both unheated and heat-inactivated bacteria. Experiments have shown that this is due to the decomposition of ergothioneine during distillation in alkaline solution. The amount of volatile base formed from stachydrine,
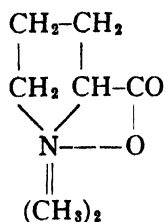
$$\begin{array}{c} CH_2-CH_2 \\ | \quad | \\ CH_2 \ CH-CO \\ \diagdown \diagup \quad | \\ N----O \\ \| \\ (CH_3)_2 \end{array}$$

TABLE I. Volatile base formation from different

Nature and concentration of organic base employed	Theor. amount of volatile base N (in γ)	Dry wt. bact. per expt. (mg.)	CULTURE 1			
			Unheated bacteria		Heated bacteria	
			Volatile base N recovered (in γ)	Per cent recovery	Volatile base N recovered (in γ)	Per cent recovery
Trimethylamine oxide 0.1 M	1400	16	1411/1414} 1415	101	2.5/3.1} 2.8	0.20
Triethylamine oxide 0.0815 M	1141	11	1122/1154} 1138	98.8	1.4/0.6} 1.0	0.08
Tri-n-propylamine oxide 0.051 M	714	18	16.8/17.4} 17.1	2.4	1.9/1.7} 1.8	0.25
Betaine 0.05 M	700	16	1.7/1.8} 1.8	0.25	1.8/2.4} 2.1	0.30
Choline 0.05 M	700	16	2.5/2.2} 2.4	0.34	2.2/2.8} 2.5	0.36
Acetylcholine 0.05 M	700	16	2.8/3.1} 3.0	0.42	2.2/2.8} 2.5	0.36
Ergothioneine 0.025 M	550	16	24.5/23.8} 24.2	6.9	24.9/24.4} 24.7	7.1
Stachydrine 0.025 M	550	16	3.1/2.5} 2.8	0.80	2.1/2.1} 2.1	0.60
			CULTURE 18			
Trimethylamine oxide 0.1 M	1400	12	1097/1125} 1110	79.2	4.2/3.6} 3.9	0.28
Triethylamine oxide 0.0815 M	1141	15	910/970} 940	82.5	3.5/2.8} 3.2	0.28
Tri-n-propylamine oxide 0.051 M	714	15	21.5/19.6} 20.8	2.9	2.2/1.7} 2.0	0.28
Betaine 0.05 M	700	12	4.2/3.6} 3.9	0.56	4.6/2.8} 3.7	0.53
Choline 0.05 M	700	12	4.6/2.8} 3.7	0.53	4.9/5.9} 5.4	0.72
Acetylcholine 0.05 M	700	12	4.2/4.2} 4.2	0.60	4.2/4.8} 4.5	0.64
Ergothioneine 0.025 M	550	12	28.3/26.3} 27.3	7.8	24.5/27.2} 25.9	7.4
Stachydrine 0.025 M	550	12	2.8/4.9} 3.9	1.1	3.5/5.6} 4.6	1.3
			CULTURE 22			
Trimethylamine oxide 0.1 M	1400	17	1592/1586} 1589	99.3	4.2/3.5} 3.9	0.28
Triethylamine oxide 0.0815 M	1141	11	1170/1140} 1155	101	1.4/1.4} 1.4	0.12
Tri-n-propylamine oxide 0.051 M	714	10	123/125} 125	17.2	1.4/1.7} 1.6	0.22
Betaine 0.05 M	700	17	2.0/2.0} 2.0	0.29	1.7/2.5} 2.1	0.30
Choline 0.05 M	700	17	2.2/2.8} 2.5	0.36	2.2/2.5} 2.4	0.34
Acetylcholine 0.05 M	700	17	2.1/2.7} 2.4	0.34	2.8/2.2} 2.5	0.36
Ergothioneine 0.025 M	550	17	21.0/23.2} 22.1	6.3	23.5/22.4} 23.0	6.6
Stachydrine 0.025 M	550	17	5.6/4.5} 5.0	1.4	4.2/5.0} 4.6	1.3

substrates by the triamineoxidease of intact bacterial cells.

Nature and concentration of organic base employed	Theor. amount of volatile base N (in γ)	CULTURE C 2					
		Dry wt. bact. per expt. (mg.)	Unheated bacteria		Heated bacteria		
			Volatile base N recovered (in γ)	Per cent recovery	Volatile base N recovered (in γ)	Per cent recovery	
Trimethylamine oxide 0.1 M	1400	15	1405 1410 } 1408	101	4.2 2.2 } 3.2	0.23	
Triethylamine oxide 0.0815 M	1411	13	1142 1135 } 1139	99.8	2.1 2.5 } 2.3	0.20	
Tri-n-propylamine oxide 0.051 M	714	14	122 123 } 122	17.2	2.2 2.5 } 2.4	0.34	
Betaine 0.05 M	700	15	2.0 2.2 } 2.1	0.30	2.0 2.0 } 2.0	0.29	
Choline 0.05 M	700	15	1.9 2.9 } 2.4	0.34	1.9 2.1 } 2.0	0.29	
Acetylcholine 0.05 M	700	15	3.1 2.8 } 3.0	0.43	4.2 3.1 } 3.7	0.53	
Ergothioneine 0.025 M	350	15	26.3 25.5 } 25.9	7.4	27.7 25.7 } 26.7	7.6	
Stachydrine 0.025 M	350	15	3.1 2.8 } 3.0	1.2	3.4 2.5 } 3.0	1.2	
		CULTURE C 3					
Trimethylamine oxide 0.1 M	1400	19	1392 1408 } 1400	100	2.8 3.5 } 3.2	0.23	
Triethylamine oxide 0.0815 M	1411	19	1120 1150 } 1135	99.4	1.1 2.5 } 1.8	0.16	
Tri-n-propylamine oxide 0.051 M	714	12	118 120 } 119	16.7	1.7 2.2 } 2.0	0.28	
Betaine 0.05 M	700	19	2.5 3.1 } 2.8	0.40	2.8 2.8 } 2.8	0.40	
Choline 0.05 M	700	19	3.2 4.6 } 3.9	0.56	2.9 3.4 } 3.2	0.46	
Acetylcholine 0.05 M	700	19	2.9 3.2 } 3.1	0.44	2.5 2.8 } 2.7	0.39	
Ergothioneine 0.025 M	350	19	25.8 26.3 } 26.1	7.5	26.6 25.9 } 26.3	7.5	
Stachydrine 0.025 M	350	19	4.2 2.8 } 3.5	1.0	2.8 4.5 } 3.7	1.1	
		CULTURE 4					
Trimethylamine oxide 0.1 M	1400	9	1124 1142 } 1133	80.8	3.1 3.9 } 3.5	0.25	
Triethylamine oxide 0.0815 M	1141	7	189 199 } 194	17.0	2.8 3.0 } 2.9	0.25	
Tri-n-propylamine oxide 0.051 M	714	8	31.1 29.7 } 30.4	4.5	2.5 2.5 } 2.5	0.35	
Betaine 0.05 M	700	9	2.2 4.5 } 3.4	0.49	2.8 2.5 } 2.7	0.39	
Choline 0.05 M	700	9	4.5 4.5 } 4.5	0.64	3.4 4.4 } 3.9	0.56	
Acetylcholine 0.05 M	700	9	3.1 3.1 } 3.1	0.44	3.5 3.5 } 3.0	0.43	
Ergothioneine 0.025 M	350	9	24.9 25.2 } 25.1	7.2	25.5 26.6 } 26.1	7.5	
Stachydrine 0.025 M	350	9	4.6 4.6 } 4.6	0.76	4.9 3.9 } 4.4	0.80	

was also insignificant, and was approximately identical in the case of both un-heated and heat-inactivated organisms.

NATURE OF VOLATILE BASE FORMED

Experiments were undertaken in order to ascertain whether activation of the triamine (trialkylamine) oxides being investigated resulted in the formation of the corresponding volatile triamine.

Erlenmeyer flasks (125 ml.) were plugged and sterilized. To each flask was added 20 ml. of washed cells of culture 22 (175 mg. dry wt. of bacteria); 20 ml. of 0.2 M phosphate buffer pH 7.0; 20 ml. of a solution containing equal portions of 0.1 M glucose and 0.1 M sodium lactate (oxidizable substrate), and 20 ml. of an approximately 0.1 M solution of the triamine oxide being studied (*vide infra*). The usual aseptic conditions were observed in these experiments. The reaction mixtures were incubated at 25°C. for about 24 hours under anaerobic conditions, using partial vacuum and alkaline pyrogallol to absorb the oxygen. The contents of each flask were examined separately as follows.

Trimethylamine oxide (20 ml. of an 0.1 M solution used). The solution was washed into a small Claissen flask, 2 ml. of strong formaldehyde and 5 ml. of saturated potassium carbonate solution were added, and approximately 10 ml. of distillate were collected in 10 ml. of ice-cold 6 per cent hydrogen peroxide by distilling *in vacuo* at about 60°C. The resulting solution was permitted to stand for one day at room temperature, and was then evaporated to a thick syrup, first over sulphuric acid and finally over phosphorus pentoxide. The syrup (150 mg.) was dissolved in 1 ml. of absolute ethyl alcohol, 10 ml. of dry, re-distilled ether were added and the supernatant liquid was decanted from the resulting precipi-tate. To the white gummy residue (127 mg.) 2.5 ml. of a saturated aqueous solution of picric acid were added. A crystalline picrate separated in yellow needles. After cooling to about 0°C. the precipitate was collected on a small filter and was dried for a short time at 110°C., 34 mg. of crystals being obtained, M.P. 194-195°C. On mixing with crystals of trimethylamine oxide picrate (M.P. 195-196°C.) a mixed M.P. of 195° was obtained. The yield of picrate was very low, being only 5.6 per cent of that expected by theory. Probably factors such as the loss of trimethylamine during distillation *in vacuo*, the incompleteness of oxidation of the very dilute solution of the base in the hydrogen peroxide solution and the solubility of the picrate in water were in part responsible for the low recovery.

Triethylamine oxide (20 ml. of 0.091 M. solution used). The procedure adopted for recovering the base was practically identical with that employed in recovering the methyl analogue. In this case the picrate was known to be less soluble in water, so the distillate was merely concentrated to approximately 10 ml., 20 ml. of a saturated aqueous solution of picric acid were added, and the solution cooled to about 0°C. The crystalline picrate which formed was dried and weighed as in the case of the methyl compound, a yield of 188 mg. being obtained. The filtrate from the first crop of crystals was concentrated to about 12 ml. over sulphuric acid, and more crystals (64 mg.) were isolated. Both frac-tions melted at 161 to 162°C. When mixed with triethylamine oxide picrate,

M.P. 164 to 166°, a mixed melting point of 163° resulted. The total yield of picrate thus obtained (252 mg.) was 41 per cent of that expected, presuming complete reduction of triethylamine oxide in the solution and no loss in recovery.

Tri-n-propylamine oxide. Several attempts were made to recover tri-n-propylamine by methods similar to those followed in the case of the methyl and ethyl oxides, but without success. The reason for this failure is undoubtedly because tri-n-propylamine oxide is but feebly reduced in comparison with its methyl and ethyl analogues. This fact is shown well in table III. By using very much larger proportions of reagents it might be possible to obtain sufficient tri-n-propylamine for purposes of identification. It is important to note in this connection that the characteristic odour of tri-n-propylamine is evident when reaction mixtures such as those employed in these experiments are made alkaline. There would seem little doubt, therefore, that tri-n-propylamine is the product of bacterial reduction of the corresponding oxide.

UNITY OF ACTIVATING ENZYME

Experiment 1. The following solutions were added to each of twelve Thunberg tubes: 1 ml. of a suspension of culture 22 (9 mg. dry wt. of bacteria), 1 ml. of 0.2 M phosphate buffer pH 7.0, 1 ml. of a solution containing equal portions of 0.1 M glucose and 0.1 M sodium lactate, and 2 ml. of triamine oxide solution. The amount and kind of triamine oxide was varied, three tubes being prepared in each case (see table II).

TABLE II. Volatile base formation from trimethylamine oxide, triethylamine oxide, and from a mixture of these compounds.

Triamine oxide employed and its concentration (5 ml. of solution)	Theoretical amount (in γ) of volatile nitrogen for complete reduction of the oxide	Volatile nitrogen recovered (in γ) after:		
		$3\frac{1}{2}$ hr.	$4\frac{1}{2}$ hr.	6 hr.
Methyl, 0.02M	1400	685	926	1115
Methyl, 0.04M	2800	660	915	1130
Ethyl, 0 02M	1400	701	940	1150
Methyl, 0 02M plus Ethyl, 0.02M	2800	693	910	1138

The tubes were evacuated and placed in a 25°C. thermostat, the amount of volatile triamine nitrogen being determined at intervals on the whole contents of one tube of each of the four varieties. From the results given in table II it will be seen that in this experiment the enzyme was saturated with respect to its substrate in 0.02 M trimethylamine oxide solution, there being no increase in volatile base formation with 0.04 M trimethylamine oxide; also the amount of volatile base nitrogen formed from triethylamine oxide was about the same as that formed from the trimethylamine oxide. If different enzymes were concerned in the activation of these two oxides, the amount of volatile base formed in the case of the solution containing both trimethylamine oxide and triethy-

lamine oxide would be expected to be approximately double that formed in a solution of corresponding concentration of either one of these compounds separately. In this experiment the amount of volatile base is about the same in the mixed solution of oxides as it is in the case of a single oxide, and it must therefore be assumed that the same enzyme is responsible for the activation of both trimethylamine oxide and triethylamine oxide.

Experiment 2. The technique adopted was identical with that followed in the foregoing experiment, using 0.02 M trimethylamine oxide, 0.02 M tri-n-propylamine oxide, and a suspension of culture 22 containing 7 mg. dry weight of bacterial cells per experiment. The results, recorded in table III, show that the propyl oxide is activated to a very much smaller extent than is the methyl oxide, only 2.4 per cent as much volatile nitrogen being recovered over a five-hour period. Also, the amount of volatile base nitrogen formed in the case of the mixed solution of trimethylamine oxide and tri-n-propylamine oxide was actually less than that formed from the methyl oxide alone. This may be due to a toxic effect exerted by the propyl radicals, to the fact that the enzyme was saturated with an excess of propyl oxide, to the fact that the adsorption of the propyl oxide by the enzyme hindered the activation of the methyl oxide, or to some similar cause. Until further work is done in order to determine the affinity of this enzyme for the substrates in question it will be imposssible to state definitely whether the propyl oxide is activated by the same enzyme as the methyl oxide.

TABLE III. Volatile base formation from trimethylamine oxide, tri-n-propylamine oxide, and from a mixture of these compounds.

Triamine oxide employed and its concentration (5 ml. of solution)	Theoretical amount (in γ) of volatile nitrogen for complete reduction of the oxide	Volatile nitrogen recovered (in γ) after 5 hrs.
Methyl, 0.02M	1400	512, 496 — 504
N-propyl, 0.02M	1400	12.3, 11.9 — 12.1
Methyl, 0.02M plus N-propyl, 0.02M	2800	471, 451 — 461

DISCUSSION

The experiments herein described show that the enzyme which activates trimethylamine oxide exhibits a well-defined substrate specificity. Thus it will activate at least the lowest three members of the homologous series of compounds having the general structure $R_3N=O$, which might be regarded as o-alkyl dialkyl hydroxylamines, $R-N{<}^{O-R}_{R}$. On the other hand compounds which possess the $(CH_3)_3N{<}^{O-}_{C\equiv}$ grouping are not activated. As yet no attempt has been made to ascertain whether other alkyl-substituted hydroxylamines, such as $\alpha\beta$-diethyl, $\beta\beta$-diethyl and $\beta\beta$-dipropylhydroxylamines are activated with the forma-

tion of volatile base. Shewan (1938) has found small amounts of dimethylamine in spoiling fish muscle, but since attempts to prepare a $\beta\beta$-dimethylhydroxylamine failed (Dunstan and Goulding 1899b) it seems unlikely that this compound is the precursor of dimethylamine.

Experiments have strongly indicated that the enzyme which activates trimethylamine oxide is the same as that which activates triethylamine oxide. So far attempts to prove that the same enzyme activates tri-n-propylamine oxide have failed, but the fact that the six different cultures all activated the three oxides to about the same degree makes it highly probable that one enzyme is involved. In view of these findings, and the specificity of the enzyme toward triamine oxides, the name "Triamineoxidease" appears to be the most fitting designation for the activating principle.

The results described in this paper are of interest in that Beatty (1938), basing his findings on the fact that Lintzel's chemical method of reduction (1934) does not cause volatile base formation from choline, betaine, γ-butyrobetaine and carnitin, stated that at least 94 per cent of the trimethylamine in spoiling fish muscle arises from trimethylamine oxide. He also showed qualitatively that trimethylamine is formed during the bacterial spoilage of fish muscle press juice.

It must be emphasized in closing that all the experiments described have been carried out under anaerobic conditions. It is not unlikely that the breakdown of certain of the bases employed would follow a different course under aerobic conditions.

SUMMARY

The bacterial enzyme previously described (Tarr 1939, a and b) which activates trimethylamine oxide to form the reduction product, trimethylamine, also activates triethylamine oxide and tri-n-propylamine oxide with formation of the corresponding volatile bases. It does not activate the $(CH_3)_3N{<}^{O-}_{C\equiv}$ group of betaine, choline, acetylcholine or ergothioneine; nor the $(CH_3)_2N{<}^{O-}_{CH_2-}{-}CH{<}$ group of stachydrine.

In view of these results, and the specificity of this enzyme toward triamine oxides, it is suggested that it be named Triamineoxidease.

Triamineoxidease exists in the cells of bacteria from widely different sources, including spoiling fish muscle, well water and surface taint butter. It has been demonstrated in organisms from five different genera.

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A FLUID SYSTEM FOR TRANSFERRING HEAT OVER SMALL TEMPERATURE GRADIENTS WITHOUT FORCED CIRCULATION[1]

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Abstract

An enclosed system of piping partly filled with liquid ammonia was found to transfer useful quantities of heat to a bunker containing a solid refrigerant (ice), with temperature gradients of 30 to 50° F. without the use of forced circulation. The system could be adapted to reduce spatial temperature variations and provide thermostatic control where solid refrigerants are used, as in railway refrigerator cars. Such arrangements are discussed briefly.

Introduction

In spite of the recent advances in mechanical refrigeration, such solid materials as ice, ice-salt mixtures, and dry ice are still used for preserving foodstuffs where suitable power sources are lacking or where economic considerations intervene. Such applications include the cooling of products in rural areas and railway car refrigeration. Under these conditions, temperature control is usually difficult since the mechanical refrigerator and its accessories, namely, distributed coils, circulating fans, and thermostats are lacking. It appears that the advantages of distributed cooling coils under thermostatic control could be applied to ice cooled systems under certain conditions, where a method capable of transferring sufficient heat from the space to the ice bunker could be devised. The present investigation was undertaken to determine the capacity of an enclosed system, partly filled with a volatile liquid (ammonia), to transfer heat.

The conditions in the end-bunker type of railway car may be described as indicating the problems involved. The bunker is filled with broken ice through which the air circulates and the resulting liquid drains from the bottom. In order to obtain car temperatures of 32 to 35° F. during the summer months it is necessary to add up to 15% salt to the ice, and under these conditions the temperature of the cooling mixture may reach 0° F. or lower. The air temperatures prevailing in the loaded car may then attain the following extremes: air temperature near bottom of bunker, 28 to 32° F.; above the load near the centre of the car, 45 to 50° F. Comparable figures are reported by several investigators (1, 2, 3). These figures indicate that a temperature difference of 50° F. may prevail between the warmest region in the car and the temperature of the cooling medium.

Recent developments have been of material assistance in reducing these spatial variations in temperature within the loading space. Cars with over-head bunkers are a big improvement (2, 6, 7) but the end-bunker type will necessarily be in use until replaced by the newer equipment. Forced air circulation by means of fans has been shown to improve conditions (1, 4) but is not yet in general use. Indirect cooling systems using brines or volatile liquids, such as that described in this study, have been used experimentally for controlling the temperature in railway cars cooled with dry ice (3, 5). Efficient operation of such systems might reasonably be expected over the relatively large temperature difference prevailing when dry ice is used as a refrigerant. When ordinary ice is used the temperature gradient would seldom exceed 50° F. and it might be reduced to 30° F. Under these con-ditions it seems reasonably certain that gravity circulation of a brine, which changes in density by only 0 3% over a 20° F. difference in temperature, could not accomplish the desired heat transfer at the levels prevailing in a railway car.

If an enclosed piping system were partly filled with a volatile liquid such as ammonia, heat could be transferred by evaporation in a "hot" coil and condensation in a "cold" coil. By analogy with mechanical systems the "hot" and "cold" coils may be termed the "evaporator" and "condenser" respectively. Since the latent heat of liquid ammonia is 545 B.t.u. per lb. at 30° F., this quantity of heat would be transferred for each pound of liquid evaporated. Since the vapour pressure of ammonia increases about 1 lb. per sq. in. for each °F. rise in temperature, between 20 and 30° F., it is obvious that a potentially adequate circulating pressure will prevail with a small difference between the temperature of the liquid in the evaporator and that of the liquid in the condenser. Similar conditions apply to other volatile fluids, but the favourable properties of liquid ammonia combined with its low cost appeared to justify tests with this material.

Description of Apparatus and Method

Valid estimates of the capacity of such systems, involving heat transfer surfaces exposed to convection cooling and connecting piping to resist fluid circulation, can be obtained only from tests conducted with equipment approxi-mating the scale that would be used in practice. For this reason the test equipment was set up on a scale similar to that required in a railway car, as shown in Fig. 1. Since the amount of heat to be transferred to improve conditions in the railway car, or for other duties, was unknown, the apparatus was designed to transfer about 1000 B.t.u. per hr. The temperature difference across each coil was assumed to be 10° F. or an over-all temperature gradient of 20° F. plus the difference between the temperatures of the liquid on the hot and that on cold coils necessary to produce circulation. The heat transfer coefficient K was taken as 2 for the evaporator and 5 for the condenser in the ice bunker. The surface areas of the coils as constructed were as follows: evaporator, 58 sq. ft.; condenser, 18 sq. ft. Both coils had an over-all vertical
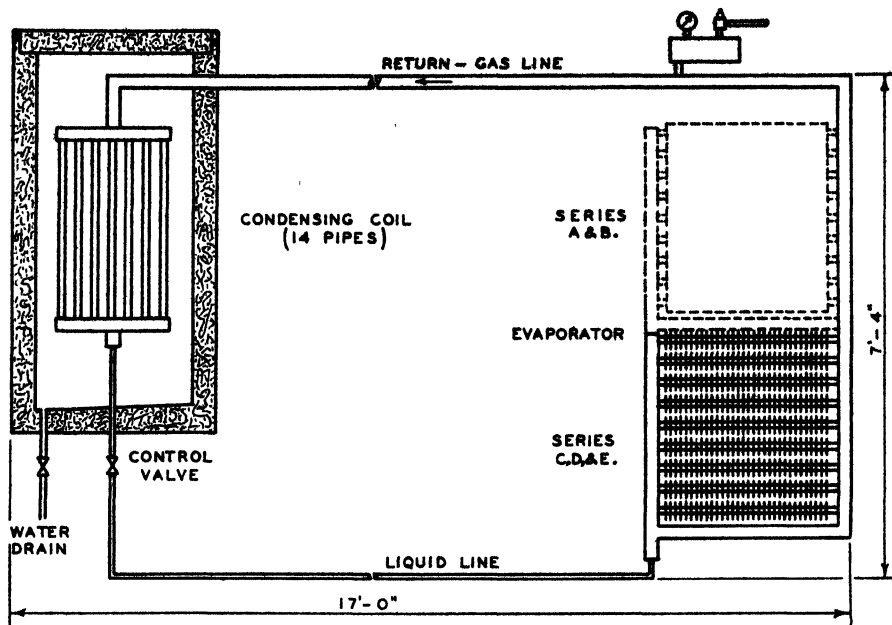
FIG. 1. *Diagram of apparatus showing positions of the evaporator in different series.*

height of 36 in.   The condenser was made up of vertical pipes welded into horizontal manifolds, while the evaporator was made up of horizontal finned pipes welded into vertical manifolds.

Further details of construction, and the general experimental arrangement, are evident from Fig. 1.   The coils were connected by ⅜-in. pipe below the liquid level, and by 2-in. pipe above the liquid level.   The condenser coil was placed in an insulated, water-tight bunker which was filled with ordinary water ice of about the same size as that used in railway cars.   The evaporator coil was hung in a large room at about 80° F. since this gave an over-all temperature difference of about 50° F. between the hot and cold regions. Suitable equipment was provided for determining the liquid level, the total pressure, the temperature of the room, and that of the several heat exchange surfaces.   A valve was also provided in the small "liquid" line to stop circulation during "blank" tests.

The method of test consisted of weighing the amount of ice melted, by draining the bunker over known time intervals. An individual test usually extended over a 48- to 72-hr. period, the amount of ice melted being determined over 6- to 8-hr. intervals, and the charge of ice tamped or supplemented as necessary.   Other observations were made more frequently. Blank tests were first conducted with the circulation shut off to determine the heat leakage through the bunker and other parts of the system.   A similar test was then made with the ammonia in circulation and the net heat transfer of the system was determined by difference.   The amount of ice melted by heat leakage yielded remarkably similar results throughout the course of the tests.

Results

The results of these investigations are reported in Fig. 2 and Table I.   In the first two series of experiments the condenser and evaporator coils were on the same level, and the effect of the ice level in the bunker, and the liquid ammonia level in the system, were determined under these conditions.   It is evident from both the figure and the table that the quantity of heat transferred was rather low.   Fig. 2 shows that the capacity of the system did not decrease until the ice level fell to a point about 12 in. below the top of the condenser. Heat conduction along the vertical pipes in the condenser may explain why a decrease in capacity did not occur at an earlier stage.

TABLE 1

Series of experiments	Number of experiments per series	Position of evaporator in relation to condenser	Liquid level in 36-in evaporator and 36-in. condenser		Average height of ice on 36-in condenser in ice bunker	Average room temp., °F	Average evap. temp , °F.	Net heat transferred by the system, B.t u.
			Condenser	Evaporator				
A	4	Vertical and at same level	18 in	18 in.	24 in or more to covering	65	60	220
B	4	Vertical and at same level	9 in.	9 in.	24 in or more to covering	63	56	320
C	5	Vertical and below cond.	Empty	Full	24 in or more to covering	80	61	840
D	6	Vertical and below cond.	Empty	Full	Covered with ice	78	—	865
E	2	Vertical and below cond.	Empty	Full	Covered with ice and water	85 5	55 5	2900
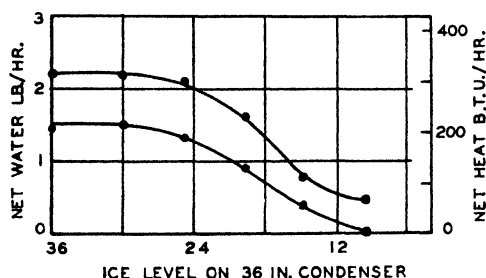
FIG. 2. *Effect of ice level around the condenser on heat transfer (Series A, lower curve; Series B, upper).*

When both coils are on the same level, the effective heat transfer surface of each coil is affected by the liquid level.   In the condenser the space occupied by the liquid is not available for condensing the gaseous phase, while the majority of the heat transfer in the evaporator takes place below the liquid level.   In Series A both coils were half-filled, and in Series B, one-quarter filled with liquid.   Table I shows that the heat transfer was greater in B. Since this occurred under conditions that decreased the capacity of the evaporator coil, but increased the capacity of the condenser, it must be concluded that the condenser coil was the factor limiting the capacity of the

entire system.   Although it is desirable to maintain the cooling surface at the highest possible level in the loading space, it is evident from the above that the evaporator must be below the level of the condenser in order to obtain the full capacity of both coils.

The evaporator coil was then lowered to a point below the condenser coil, and the system charged with liquid ammonia to fill the evaporator coil only. This arrangement permitted both coils to operate at full capacity.   Comparison of the net heat transfer in Series *B* and *C* shows that this arrangement increased the heat capacity about 500 B.t.u. per hr., part of which may be attributed to an increase in room temperature.

In the experiments reported under Series *D*, the finned evaporator was enclosed in a vertical duct constructed from insulating board.   This arrangement facilitated a downward movement of the warm air from the higher levels and should tend to offset the disadvantages of lowering the cooling coil.   The results showed that the addition of this duct had no effect on the capacity of the system, the amount of heat transferred being essentially the same as in Series *C*.   A number of thermocouples placed in the duct above and below the evaporator showed that the air was cooled 11.3° F. in one passage over the coil, and that the volume of air circulated was about 70 cu. ft. per min.

In Series *C* and *D* it was observed that the surface temperature of the evaporator varied by as much as 10° F. from time to time, and the net heat transferred in different experiments under the same conditions varied from 650 to 1040 B.t.u. per hr.   These variations were finally traced to the condition of the ice in the bunker, heat transfer being greatest for a short period after tamping the ice, and then gradually diminishing to the lower values which prevailed over the greater part of the experimental period.   This behaviour indicated that the capacity might be increased by improving the conditions for heat transfer between the coil and the ice in the bunker.   It was evident that this could be accomplished by allowing the water from the melting ice to accumulate in the bunker.

The final series of experiments was conducted in this way.   Experimentally it proved simpler to fill the bunker with a known quantity of ice and add water at 32° F. at the start of an experiment and determine the quantity of each remaining at the end of the test.   The net heat transfer in these experiments was 2900 B.t.u. per hr. as shown in Table I.   Other measurements showed that the mean temperature of the ice and water mixture was 37° F., that the temperature of the air leaving the evaporator was 18° F. lower than that of the air entering the duct, and that approximately 150 cu. ft. per min. of air was circulated over the evaporator.

These results were obtained with a prevailing temperature difference, between the cooling medium and the air surrounding the evaporator, of about 50° F.   As mentioned before, this is apparently the condition prevailing in end-bunker railway cars under certain conditions.   In order to be of value the temperature in the warm region should be reduced about 20° F., a con-

dition that would reduce the over-all temperature difference to about 30° F. Assuming a linear relation between heat transferred and temperature difference, the results obtained in Series E indicate that a capacity of about 1750 B.t.u. per hr. would be obtained with the present system over a temperature difference of 30° F.   In designing the system for about 1000 B.t.u. per hr., an over-all temperature gradient of 20° F. was assumed, and the above results indicate a capacity of 1150 B.t.u. per hr. under these conditions.

In these experiments no provision was made for measuring the actual liquid temperature within the condenser and evaporator. However, the surface temperature of the liquid line leaving the bunker and the surface temperature of the evaporator were essentially the same within the error of measurement under the conditions prevailing in these tests.   From this and the observed performance, it appears that the necessary circulation pressure was provided by a relatively negligible difference in the temperature of the liquids in the hot and cold coils.   Other observations indicate that the differences between the temperature of the liquid in the condenser and that of the ice and water in the ice bunker, and between that of the liquid in the evaporator and the mean effective air temperature, were approximately equal.

Discussion

The results show that a system of this sort is capable of transferring useful quantities of heat over relatively small temperature differences without the use of forced circulation.   In order to avoid the need for an excessively large condensing coil where water ice is used, it is necessary to allow the water to accumulate in the bunker to improve heat transfer from the coil.   When solid carbon dioxide is used the difference between the temperature of this solid and that of the coil is so much greater that the addition of a non-freezing liquid to facilitate heat transfer may be unnecessary.

A system of this sort may be found useful for the control of temperature, or for reducing spatial variations in temperature in applications where sources of power are lacking.   The reduction in spatial temperature variations is accomplished by the use of coils that can be suitably located and distributed throughout the space.   The value of such a system for improving conditions in end-bunker railway cars depends on a number of other factors which have not been studied and need not be discussed here.

When dry ice is used for cooling, excessively low temperatures may result, with consequent waste of refrigerant and product deterioration, unless some means of temperature control is provided.   Water ice will seldom produce detrimentally low temperatures but may result in temperatures lower than can be economically maintained under certain conditions, e.g., rural egg grading and packing stations.   The use of the present indirect system would permit the ice to be placed in a well insulated bunker to reduce wastage and also permit thermostatic control of the temperature in the space.

Thermostatic control would consist of a sensitive element acting on a suit-able closure member in either the liquid or gas line, to modulate or stop the circulation.   In this connection the maximum pressure to be overcome need only be that of the head of liquid in the condenser.   Observations made during the course of the present experiments indicate that the temperature in the gas line is essentially that of the surrounding space.   This suggests placing the sensitive element in the gas line.   The closure member should also be placed in the gas line since the excess pressure developed in the evaporator would return the liquid already in the evaporator to the condenser through the liquid line.   Closing the liquid line would permit appreciable heat transfer following closure, since the liquid in the evaporator would have to distil over into the condenser coil with consequent absorption of heat.

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CANADIAN WILTSHIRE BACON

XII. EFFECT OF HEAT TREATMENT ON THE COLOUR AND COLOUR STABILITY OF BACON[1]

BY C. A. WINKLER[2] AND J. W. HOPKINS[3]

Abstract

The colour of quadruplicate pieces of factory-cured bacon heated at temperatures of 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr. was measured at the conclusion of heating, and also after subsequent exposure of the samples for 12, 20, and 96 hr. at 10° C. and 95% relative humidity. Interacting effects of time and temperature of heating on colour at the conclusion of treatment were demonstrable. At 40 and 50°, total intensity increased with the duration of heating; at 60 and 70°, there was no definite trend, and at 80° it diminished markedly as the period of heating was prolonged. The maximum average intensity resulted from heating at 70°.

The decrease in intensity after 12 hr. exposure was related to the duration, but not to the temperature, of previous heating. There was no significant change in intensity between 12 and 20 hr., but a further decrease was evident at the end of 96 hr. The decrease in green intensity was still related to the duration, rather than to the temperature, of heat treatment, but the effects of duration of heating on red and blue stability, noted at 12 and 20 hr., were now replaced by temperature effects.

Partial correlation coefficients indicate that increased nitrite content of the meat at the conclusion of heating tended to be associated with a lower intensity of colour. On the other hand, both nitrite content and loss in weight (chiefly moisture) on heating were correlated with increased colour stability on exposure.

In the preceding paper of this series (2), White, Cook, and Winkler describe the details of an experiment designed to investigate the effect of heating Wiltshire bacon, as in smoking, for various periods at different temperatures, on its nitrite content and colour. The experiment comprised four periods of heating, namely, 5, 10, 20, and 40 hr., and six temperatures, 20, 40, 50, 60, 70, and 80° C., or 24 treatments in all. Four out of 16 "backs" cut from individual factory-cured sides were allotted at random to each of the four periods of heating, and each back was subdivided into six portions which were allocated, also by a random process, to the six temperatures maintained for each period of time. The above-mentioned authors conclude that heating at 55° C. resulted in an approximately normal nitrite content. Temperatures

below 55° resulted in increased amounts of nitrite being found on analysis, whereas higher temperatures diminished the concentration of this constituent. These effects of temperature were accentuated by prolonging the time of heating.

It is now desired to describe the results of colour measurements of the 96 individual samples after treatment, secured by means of the photoelectric comparator employed in other phases of this investigation, already described elsewhere (3). The effect of heat treatment on colour and colour stability will first be dealt with, after which the correlation between these colour measurements and the foregoing determinations of nitrite content will be discussed.

Effect of Heat Treatment on Colour

The photoelectric comparator was employed as before to determine the component intensities of red, green, and blue light, relative to the white standard, reflected or scattered from the surface of the cut samples (4), and the sum of these three relative values was again taken as the index of colour intensity or total brightness.

Intensity or Total Brightness

Table I shows the results of an analysis of the variance (1) of the colour intensity as defined above, at the conclusion of heating of the 80 individual samples treated at temperatures of 40 to 80° C., and Table II the treatment averages, as well as the means by periods, for all temperatures, and by temperatures, for all periods. As was explained by White, Cook, and Winkler (2) the effect of temperature, which is deduced from comparisons between sub-samples of the same sides, is more accurately determined than that of period of heating, which is affected by the variability between sides. This is reflected in the magnitude of the mean square errors *(a)* and *(b)* in Table I.

The analysis of variance demonstrates a highly significant effect of temperature on subsequent intensity of colour, and reference to Table II will indicate that the mean intensity, averaged for all periods, increased with

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR TOTAL BRIGHTNESS
OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square
Period of heating (all temps.)	3	261.0
Error *(a)* (between sides)	12	303.8
Temperature (all periods)	4	3,273.0***
Interaction, temp. × period	12	618.6***
Error *(b)* (within sides)	45	39.0

***Exceeds mean square error, 0.1% level of significance.*

TABLE II

AVERAGE COLOUR INTENSITY OR TOTAL BRIGHTNESS OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	121	120	126	154	163	168	146
10	115	115	134	155	163	158	145
20	113	128	139	152	157	143	144
40	—	143	158	161	169	128	152
Mean	116	126	139	156	163	149	147

temperature from 40 to 70°, falling off again however at 80°. The means for the four periods of heating, when taken over all five temperatures, do not differ significantly, but there is a significant interaction of temperature and duration of heating. Thus at 40 and 50°, the samples exhibited an increasing intensity of colour as the duration of treatment was prolonged. At 60 and 70° there was no appreciable trend, and at 80° the trend was the reverse of that noted at 40 and 50°, the brightness diminishing markedly with length of heating at this temperature.

For the reasons previously stated (2), the observations made on the samples heated at 20° C. were not included in the foregoing analysis of variance. A separate examination of these data did not indicate any significant difference in the brightness of the samples held for 5, 10, and 20 hr. at this temperature.

Chroma or Colour Quality

Tables III, IVA, IVB, and IVc give the analyses of variance and average values of the component red, green, and blue intensities of the samples subjected to the various treatments, in the same form as that adopted in Tables I and II above.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		Red	Green	Blue
Period of heating (all temps.)	3	39.7	50.5	30.3
Error (a) (between sides)	12	29.9	33.3	28.9
Temperature (all periods)	4	558.3***	336.2***	404.1***
Interaction, temperature × period	12	94.5***	61.7***	15.5 ·
Error (b) (within sides)	45	9.6	5.1	11.6

***Exceeds mean square error, 0.1% level of significance.*

TABLE IV. A

AVERAGE INTENSITY OF RED COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION
OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	49.0	46.0	49.0	61.0	64.9	65.6	57.3
10	46.4	46.0	53.4	62.7	65.7	61.4	57.9
20	43.7	50.8	53.9	61.0	62.4	57.8	57.2
40	—	57.2	62.7	63.8	66.5	50.9	60.2
Mean	46.3	50.0	54.8	62.1	64.9	59.0	58.2

The analyses of variance of red and green, and the treatment averages shown in Tables IVA and IVB, both reproduce features already noted in the results for total brightness, namely an increase in the average intensity for all periods of treatment with temperature up to 70°, followed by a reduction at 80°, and a significant interaction of temperature and treatment, intensity of colour increasing with duration of heating at 40 and 50°, and decreasing at 80°. There might seem to be some difference in the behaviour of the blue component, for its interaction mean square in Table III does not attain

TABLE IV. B

AVERAGE INTENSITY OF GREEN COMPONENT OF COLOUR OF BACON SAMPLES AT
CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	37.9	37.3	39.3	46.1	50.0	52.6	45.1
10	35.4	35.2	41.2	47.5	50.4	48.7	44.6
20	35.4	39.4	42.0	46.7	48.9	44.7	44.4
40	—	44.2	49.4	51.1	54.2	40.0	47.8
Mean	36.2	39.0	43.0	47.9	50.9	46.5	45.4

significance. Reference to Table IVc will, however, indicate that there is in fact some reversal of the time effect at the high and low temperatures, but that the effects of treatment in general are less pronounced in this component, and that the differences between the intensities recorded after heating for 5 and 10 hr., and between those for 20 and 40 hr., in particular, are for the most part quite insignificant. These comparisons, which contribute eight of the total 12 degrees of freedom for interaction, dilute the variance computed from the data as a whole, and when the remaining four degrees of freedom, representing the average interaction, with temperature, of heating for 5 and 10 hr. on the one hand and for 20 and 40 hr. on the other were isolated, they

TABLE IV. C

AVERAGE INTENSITY OF BLUE COMPONENT OF COLOUR OF BACON SAMPLES AT
CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–80°
	20	40	50	60	70	80	
5	33.8	36 4	37.4	47.0	47.9	49.6	43.6
10	33.3	33 8	39.7	44.9	46.6	47.4	42.5
20	34.3	37.4	42.8	44 3	46 1	35.5	41.2
40	—	42.2	45.8	46.0	48.5	37.0	43.9
Mean	33.8	37 4	41.4	45.5	47.3	42 4	42 8

yielded a mean square of 34 9, significantly in excess of the mean square error *(b)*, 11 6. It is to be concluded, therefore, that the treatments imposed influenced all three colour components in a similar manner, but that the magnitude of the effects was greatest in the red and least in the blue region.

The samples maintained at 20° C., which were again considered separately, failed to exhibit any statistically significant differences attributable to the period of heating.

Effect of Heat Treatment on Colour Stability

In addition to the foregoing observations at the conclusion of heat treatment, further measurements of the intensity of the red, green, and blue components of colour were made after the treated samples of bacon had been exposed in a light-proof chamber for 12, 20, and 96 hr., at 10° C. and 95% relative humidity. These enabled the effects of heat treatment on colour stability, which will again be considered under the separate heads of total intensity and colour quality, to be followed in some detail.

Intensity or Total Brightness

Tables V and VI summarize the results of the observations of change in total intensity as defined above, the former giving the analyses of variance (40 to 80°), and the latter the treatment averages for the 12-, 20-, and 96-hr. periods of exposure.

It will be noted at once from Table V that whereas the change in intensity after both 12 and 20 hr. exposure varied significantly in relation to the duration, but not the temperature, of heating, after 96 hr. these effects of heating period had disappeared. Curiously enough, the samples heated for the shortest periods showed the greatest decreases in intensity of colour after 12 hr. exposure (Table VI). There was no significant change in intensity between 12 and 20 hr.; in fact, the mean decrease observed after 20 hr., 5.2 units, was slightly less than the corresponding figure of 6.3 after 12 hr., but the difference is within the limits of the experimental error. By 96 hr., however, the mean decrease (at 19.0 units) was appreciably greater and, as noted above.

TABLE V

ANALYSIS OF VARIANCE OF CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON
EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	245.6***	323.3*	182.0
Error (a) (between sides)	12	18.9	58.7	60.8
Temperature (all periods)	4	11.7	9.9	135.6*
Interaction, temperature × period	12	44.0	40.3	43.2
Error (b) (within sides)	45	47.3	45.2	45.1

* *Exceeds mean square error, 5% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VI

AVERAGE CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON
SAMPLES SUBJECTED TO HEAT TREATMENT

Expo- sure, hr.	Duration of heat treat- ment, hr.	Temperature, °C.						Mean, 40–80^b
		20	40	50	60	70	80	
12	5	−10.6	−11.2	−14.4	−10.0	− 9.4	− 5.2	−10.0
12	10	− 3.2	− 2.4	−12.4	− 9.8	− 7.0	− 7.6	− 7.9
12	20	− 5.9	− 8.2	− 3.6	− 5.3	− 3.6	− 6.1	− 5.4
12	40	—	− 1.2	− 0.8	+ 1.4	− 3.0	− 5.7	− 1.9
12	Mean	− 6.6	− 5.8	− 7.8	− 5.9	− 5.7	− 6.2	− 6.3
20	5	− 7.0	− 7.2	−11.1	− 9.2	− 8.6	− 5.8	− 8.4
20	10	− 0.8	− 2.4	− 9.8	−10.6	− 8.8	− 7.6	− 7.8
20	20	− 2.3	− 5.1	− 1.4	− 7.9	− 3.8	− 7.3	− 5.1
20	40	—	− 0.8	+ 1.3	+ 5.6	− 0.8	− 3.2	+ 0.4
20	Mean	− 3.4	− 3.9	− 5.3	− 5.5	− 5.5	− 6.0	− 5.2
96	5	−15.2	−15.4	−20.7	−22.4	−22.7	−18.1	−19.9
96	10	− 7.9	− 7.6	−19.5	−21.8	−21.4	−22.2	−18.5
96	20	− 9.8	−14.8	−12.2	−18.6	−16.4	−13.7	−15.1
96	40	—	−21.0	−23.2	−22.5	−27.3	−17.8	−22.4
96	Mean	−10.9	−14.7	−18.9	−21.4	−22.0	−17.9	−19.0

the earlier effects of duration of heating had disappeared, but differences related to temperature had developed. These were such that the magnitude of the decrease in intensity increased with temperature of heating from 40 to a maximum at 70°, falling off again at 80°. There is thus a parallelism between the effects of temperature on colour at the conclusion of heating,

and on colour stability, which is worthy of remark in view of the correlation between initial intensity and stability noted elsewhere (5). In contrast to the effects on initial colour, however, there was no demonstrable interacting influence of temperature and duration of heating on stability.

Chroma or Colour Quality

Table VII gives the results of analyses of variance of the change in the component red, green, and blue intensities after exposure of the samples for 12, 20, and 96 hr. The results for the red and blue components resemble those already noted in the foregoing analysis of the changes in total intensity in indicating significant differences in stability after 12 and 20 hr. related to the duration of heating, which were replaced by differences ascribable to temperature at the end of 96 hr. Tables VIII and X further indicate that these effects were similar in nature to the ones observed in total intensity. Both of the components mentioned showed a greater reduction at 12 and 20 hr. following the shorter periods of heating, and a maximum decrease in intensity after 96 hr. in the samples heated at 70°.

TABLE VII

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR ON EXPOSURE
OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square (red)			Mean square (green)			Mean square (blue)		
		12 hr. ex-posure	20 hr. ex-posure	96 hr. ex-posure	12 hr. ex-posure	20 hr. ex-posure	96 hr. ex-posure	12 hr. ex-posure	20 hr. ex-posure	96 hr. ex-posure
Period of heating (all temps.)	3	26 6*	39.6*	23 1	18.0**	17.3	28.0*	47.6***	64.9**	22.0
Error (a) (between sides)	12	5 6	9.9	9 0	2 2	5 6	6 8	3 4	9.4	7 4
Temperature (all periods)	4	4 1	8.7	33 5*	1 7	1 3	5 6	5 8	3.0	18 4*
Interaction, tempera-ture X period	12	12.3	9 5	8 9	5.2	4.9	3.5	4.7	4.7	6 0
Error (b) (within sides)	45	7.0	4.9	8.3	6.7	6 6	6.1	3 7	6.5	6 0

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

The changes in the green component, on the other hand (Table IX), followed a somewhat different course, perceptible effects of duration of heating persisting even after exposure of the samples for 96 hr., whereas temperature effects were confined to narrow limits, which did not exceed the experimental error. After 96 hr. exposure, however, the decrease in green intensity of the various samples was no longer inversely proportional to the duration of heating. There was still a progressive diminution in loss of intensity from

TABLE VIII

AVERAGE CHANGE IN RED INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO
HEAT TREATMENT

Expo-sure, hr.	Duration of heat treat-ment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−5.2	−3.2	−5.8	− 4.0	− 4.2	− 0.4	−3.5
12	10	−1.8	−1.0	−4.4	− 5.4	− 3.4	− 1.8	−3.2
12	20	−0.0	−2.6	−0.2	− 1.5	− 0.4	− 2.4	−1.4
12	40	—	−0.0	−0.4	− 0.6	− 2.2	− 3.0	−1.3
12	Mean	−2.3	−1.7	−2.7	− 2.8	− 2.5	− 1.9	−2.3
20	5	−4.1	−1.3	−4.5	− 4.0	− 4.7	− 1.8	−3.3
20	10	−1.6	−2.1	−4.8	− 5.5	− 4.8	− 3.4	−4.1
20	20	+0.6	−2.2	+0.3	− 4.4	− 2.2	− 4.1	−2.5
20	40	—	−0.6	−0.3	+ 0.8	− 1.7	− 2.2	−0.8
20	Mean	−1.7	−1.6	−2.3	− 3.2	− 3.4	− 2.9	−2.7
96	5	−7.7	−5.8	−7.9	− 8.9	− 9.9	− 7.2	−7.9
96	10	−4.4	−4.2	−8.3	−10.5	−10.8	−10.9	−8.9
96	20	−3.4	−7.0	−5.0	− 9.3	− 7.4	− 6.7	−7.1
96	40	—	−8.9	−9.2	− 9.6	−11.9	− 8.2	−9.5
96	Mean	−5.2	−6.5	−7.6	− 9.6	−10.2	− 8.2	−8.4

TABLE IX

AVERAGE CHANGE IN GREEN INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED
TO HEAT TREATMENT

Expo-sure, hr.	Duration of heat treat-ment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−4.1	−3.9	−3.3	−1.6	−2.5	−2.1	−2.7
12	10	−0.6	−0.4	−3.6	−2.2	−1.9	−1.6	−1.9
12	20	−2.4	−3.2	+0.1	−1.7	−0.6	−1.8	−1.4
12	40	—	−0.5	−0.5	+0.7	−0.6	−1.2	−0.4
12	Mean	−2.4	−2.0	−1.8	−1.2	−1.4	−1.6	−1.6
20	5	−2.6	−2.8	−2.2	−0.5	−0.8	−1.0	−1.5
20	10	+0.6	+0.1	−2.2	−1.8	−1.9	−0.4	−1.2
20	20	−1.4	−1.5	+0.5	−0.8	+0.2	−1.2	−0.6
20	40	—	+0.3	+1.1	+2.2	+0.2	−0.7	+0.6
20	Mean	−1.1	−1.0	−0.7	−0.2	−0.6	−0.8	−0.6
96	5	−5.2	−5.0	−5.7	−4.8	−5.9	−5.0	−5.3
96	10	−2.2	−1.9	−5.0	−5.4	−4.9	−4.1	−4.3
96	20	−3.0	−4.3	−3.0	−4.3	−3.3	−3.0	−3.6
96	40	—	−5.8	−7.0	−6.7	−7.4	−4.5	−6.3
96	Mean	−3.5	−4.3	−5.2	−5.3	−5.4	−4.2	−4.9

TABLE X

AVERAGE CHANGE IN BLUE INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED
TO HEAT TREATMENT

Expo-sure, hr.	Duration of heat treat-ment, hr.	Temperature, °C.						Mean, 40–80°
		20	40	50	60	70	80	
12	5	−1.3	−4.2	−5.2	−4.4	−2.7	−2.8	−3.9
12	10	−0.8	−1.0	−4.4	−2.3	−1.7	−4.3	−2.7
12	20	−3.5	−2.4	−3 5	−2.1	−2.6	−1.9	−2.5
12	40	—	−0.7	+0.2	+1.3	−0.3	−1.4	−0.2
12	Mean	−1.8	−2.1	−3.2	−1.9	−1.8	−2.6	−2.3
20	5	−0.2	−3.1	−4.5	−4.8	−3.0	−3.0	−3.7
20	10	+0.1	−0.4	−2.8	−3.4	−2 0	−3.8	−2.5
20	20	−1.5	−1.5	−2.2	−2.6	−1.8	−2.0	−2.0
20	40	—	−0.5	+0.5	+2 5	+0.7	−0.3	+0.6
20	Mean	−0.5	−1.4	−2.2	−2.1	−1.5	−2.3	−1.9
96	5	−2.2	−4 5	−7 1	−8.7	−6 9	−5.8	−6.6
96	10	−1.3	−1.6	−6.2	−5.9	−5.7	−7.1	−5.3
96	20	−3.4	−3.5	−4.2	−5.0	−5 6	−4.0	−4.4
96	40	—	−6 3	−7.2	−6 2	−8 0	−5.0	−6.6
96	Mean	−2 3	−4.0	−6 2	−6.5	−6 6	−5.5	−5.7

the samples heated for 5 hr. to those heated for 20 hr., but, of all samples, the ones heated for 40 hr. now showed the maximum decrease. It is possible that after still further exposure, the decrease in green intensity following the various heat treatments might eventually have conformed to that of the other two components, but a further experiment would be required to test this point.

Correlation of Colour and Colour Stability with Moisture Loss and Nitrite Content

White, Cook, and Winkler having shown (2) that the nitrite content of the various samples was significantly affected by the heat treatment to which they were subjected, it was of interest to determine the extent to which changes in colour were related to the effects of heating on nitrite and moisture contents. These authors point out that the observed loss of weight of the samples on heating was an imperfect criterion of drying, as at the higher temperatures there was also some loss of melted fat which was absorbed by the paper wrapping. In the absence of more reliable data, however, the total loss in weight was of necessity adopted as the only available index of dehydration.

Table XI shows the coefficients of simple correlation between the loss in weight and logarithmic nitrite content (2) on the one hand, and the intensity and subsequent stability of colour on the other. There was a significant positive correlation between loss in weight and the intensity of red and green at the conclusion of heating, and a negative association between nitrite content

TABLE XI

COEFFICIENTS OF SIMPLE CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT
AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	r, intensity at conclusion of heating	r, change after exposure		
		12 hr.	20 hr.	96 hr.
Loss in weight × total intensity	+.26*	+.26*	+.10	+.09
Loss in weight × red intensity	+.28**	+.06	−.04	−.19
Loss in weight × green intensity	+.29**	+.16	+.10	+.06
Loss in weight × blue intensity	+.19	+.19	+.12	−.06
Log. nitrite content × total intensity	−.34**	+.07	+.23*	+.25*
Log. nitrite content × red intensity	−.37**	+.19	+.27*	+.27*
Log. nitrite content × green intensity	−.34**	−.10	−.01	+.04
Log. nitrite content × blue intensity	−.29**	+.12	+.09	+.20

*Exceeds 5% point (r = ±0.20).
**Exceeds 1% point (r = ±0.27).

and all three colour components, the latter indicating that samples above-average in nitrite content tended to have a lower intensity of colour. When the changes in colour on exposure were considered, the only significant correlations in evidence were those between loss in weight during heating and the change in total intensity after 12 hr. exposure, and between nitrite content and the change in red and in total intensity after 20 and 96 hr. All of these coefficients are positive, indicating that above-average loss in weight and nitrite content were associated with greater stability of colour on exposure.

In this connection, however, it is again necessary to take account of the fact that there was a significant negative correlation between the loss in weight and nitrite content of individual samples, and significant positive correlations between the individual red, green, and blue intensities at the conclusion of heating, as well as between the changes in each of these components in any particular sample on exposure. For this reason, the partial correlation coefficients shown in Table XII were computed.

The partial coefficients for total intensity at the conclusion of heating suggest that the apparent correlation of this quantity with loss in weight (Table XI) was a consequence of the negative association, noted above, between loss in weight and nitrite content, the latter alone significantly affecting total brightness. There does seem to have been a significant relation between loss in weight and the individual intensities of green and blue, but as the correlation was positive in one case and negative in the other, the resultant effect on total intensity was inappreciable. Nitrite content, on the other hand, may be judged to have had a demonstrable effect on total intensity, but apparently no selective action on the individual components of colour.

The partial coefficients also bring out relations between loss in weight and nitrite content, and the change in intensity of colour after 12 hr. exposure, not apparent from Table XI. In this case, there were significant effects not

TABLE XII

COEFFICIENTS OF PARTIAL CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	Independent of	r, intensity at conclusion of heating	r, change after exposure		
			12 hr.	20 hr.	96 hr.
Loss in wt. × total intensity	Log. nitrite content	+.04	+.42**	+.36**	+.37*
Loss in wt. × red intensity	Log. nitrite content, green and blue intensity	−.06	+.30**	+.12	−.13
Loss in wt. × green intensity	Log. nitrite content, red and blue intensity	+.22*	−.28**	−.11	+.11
Loss in wt. × blue intensity	Log. nitrite content, red and green intensity	− 22*	+.39**	+.20	+.07
Log. nitrite content × total intensity	Loss in weight	− 24*	+.35**	+ 42**	+.43**
Log. nitrite content × red intensity	Loss in weight, green and blue intensity	− 16	+ 45**	+ 31**	+.14
Log. nitrite content × green intensity	Loss in weight, red and blue intensity	+ 11	−.45**	−.24*	−.13
Log. nitrite content × blue intensity	Loss in weight, red and green intensity	− 05	+.43**	+.19	+.17

*Exceeds 5% point (r = ±0.21).
**Exceeds 1% point (r = ±0.27).

only on the change in total intensity, but also on the changes in red, green, and blue, each independent of the other two. This is a feature of some interest, for which no explanation seems to be readily forthcoming. It is also to be noted that additional loss in weight and nitrite content were associated with increased stability of red and blue over the 12-hr. period, but with a decreased stability of green.

Separate effects of nitrite content on red and green stability were still detectable in the observations made after 20 hr. exposure, but by 96 hr. these independent effects were no longer distinguishable, although there was still a significant correlation between nitrite content and the change in total intensity. Loss in weight during heating was likewise correlated with the total decrease in intensity up to 96 hr., but in this instance independent effects on the individual components apparently did not persist beyond 12 hr. of exposure. Both loss in weight, which was uncorrelated, and nitrite content, which was negatively correlated with the total intensity of colour at the conclusion of heating, were associated with enhanced colour stability on subsequent exposure.

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CANADIAN WILTSHIRE BACON

XIII. TENDERNESS OF BACON AND EFFECT OF HEAT TREATMENT ON TENDERNESS[1]

By C. A. Winkler[2] and J. W. Hopkins[3]

Abstract

Measurements made on uncooked samples of two factory-cured sides from each of 22 Canadian packing plants indicated significant differences between plants in respect of tenderness. The maximum average cutting force required by the product of an individual plant was more than three times the minimum plant average. The individual results however were fairly uniformly distributed over the observed range of variation, neither the maximum nor the minimum representing an isolated extreme. Partial correlation coefficients indicated a significant influence of pH of both pump and cover pickle, in conjunction with number of "stitches" and days in cure respectively, on tenderness, but no appreciable relation of this property to salt content, moisture content, nitrate content or pH of the meat, or number of days from curing to receipt at the laboratory, was demonstrable.

A second series of observations on samples heated at 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr., as in smoking, demonstrated significant interacting effects of the temperature and duration of heat treatment on tenderness. Excepting one notably anomalous result, the general tendency was for toughness to increase with the prolongation of temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. On the average, the maximum toughness was observed after heating at 50° C.

Introduction

In the course of a study of factors affecting the quality of Canadian Wiltshire bacon (1), determinations of the tenderness of two series of samples were made by means of the apparatus designed for this purpose by one of the authors (6). The two series comprised (i) samples of two factory-cured sides from each of 22 Canadian packing plants, and (ii) samples, of the same origin as in (i), subjected to various heat treatments, as in smoking.

As indicated in a previously published description (6) the apparatus actually provides a measure of toughness rather than of tenderness, namely the work required to cut through a piece of meat of specified width and thickness. The operation of the apparatus was the same as that previously described (6), and the data presented as indices of the cutting force are again the areas under the curve traced by the recording pen of the instrument in each case, corrected for thickness of sample. As these are directly comparable, they may be regarded as "relative work", without conversion into standard energy units. It was however found that over the range encountered in this series,

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the cutting force required varied more nearly as the square than as the actual thickness of individual samples. A Least Squares solution, based on 105 observations, actually indicated an average increase of 1.82 in the logarithm of the work done per unit increase in log thickness. This relation was accordingly used, in preference to the proportional factor previously adopted (6), to correct the observations for deviations of the individual test slices from the standard thickness of 5.0 mm.

Tenderness of Commercial Sides

Determinations were made on uncooked specimens from each of the 44 sides in series (i). For this purpose a sample of the necessary size was taken from the ham muscle of each side on arrival at the laboratory, the usual precautions being observed to secure representative material free from obvious striations of connective tissue (6).

After correction for thickness, an analysis of the variance (4) of the 44 resulting determinations indicated that the differences between the averages of the two sides from individual plants were significantly greater than would be expected from the observed variability of sides from the same plant. Table I summarizes the results of these observations, from which it is to be noted that the maximum average cutting force required by the product of an individual plant was as much as three times the minimum plant average. The individual results were however fairly symmetrically distributed over the observed range of variation, neither the maximum nor the minimum being an isolated extreme.

TABLE I

SUMMARY OF MEASUREMENTS OF TOUGHNESS, CORRECTED FOR THICKNESS, ON SAMPLES FROM 44 COMMERCIAL SIDES

Toughness	Relative cutting force required
Maximum	
Individual side	4.2
Plant (average of 2 sides)	3.6
Minimum	
Individual side	0.9
Plant (average of 2 sides)	1.2
Mean	2.4

As the sides from the various plants were not all of the same age when received at the laboratory, it would be incorrect to assume that all of the variation in tenderness of product between plants was ascribable to differences in the curing practices employed. Nevertheless, in view of the relation between tenderness and pH previously observed in samples of raw pork adjusted to

various degrees of acidity and alkalinity by injections of lactic acid or ammonia solutions (6), the effect of pH of pickle (3) on the toughness of the present series of samples was investigated. Omitting four plants for which the analytical data were incomplete, statistically significant correlation coefficients of -0.58 between the cutting force required and the product of pH of pump pickle (mean of two samples) $\times$ number of "stitches", and of $+0.48$ between cutting force and the product of pH of cover pickle $\times$ number of days in cure were obtained from the data for the remaining 18. There was no appreciable correlation ($r = -0.09$) between the two acidity indices for the same plant, with the result that the partial correlation of each index with toughness, independent of the other, at -0.61 and $+0.52$ respectively, did not differ significantly from the simple value. It is to be noted that the average pH of the pump pickles, at 7.6, was on the alkaline side of neutrality, and that of the cover pickles, at 6.6, was on the acid side, but in view of the fact that the pH of the meat itself on receipt at the laboratory, although previously shown to be affected to some extent by the pH of the pickles (3), nevertheless varied only within narrow limits, and could not be demonstrably related to toughness on the number of observations available, the mode of action of the pickles in modifying this last property remains obscure.

Coefficients of correlation between toughness and salt, moisture and nitrate content (2), as well as number of days from curing to receipt at the laboratory, were also computed, but failed to indicate any significant relation.

Effect of Heat Treatment on Tenderness

The heat treatments represented in Series (ii) comprised six temperatures, namely, 20, 40, 50, 60, 70, and 80° C., and four periods of heating, 5, 10, 20, and 40 hr. The experimental procedure, which was designed primarily to study the effect of heat treatment on nitrite content and colour, and has been described in detail elsewhere (5), may be summarized as follows: Sixteen "backs" cut from individual sides were allotted at random, four to each of the four periods of heating, and a piece from each back was allocated, also by a random process, to the six temperatures maintained for each period. The individual pieces, having been trimmed free of the cut portions of the ribs and the layer of back fat, were wrapped first in lightly waxed glassine paper and then in kraft paper, and heated in ovens maintained at the specified temperatures. At the conclusion of heating, the surface layers of each piece were trimmed off prior to the selection of suitable samples for the determination of tenderness.

Table II shows the analysis of variance of the measurements on the individual pieces, and Table III summarizes the treatment averages (means of four pieces). Unfortunately, through an oversight, the pieces which should have been heated at 20° C. for 40 hr. were removed at the end of 20 hr.; and those heated at 80° C. for 40 hr. were dehydrated to such an extent that satisfactory measurements of toughness could not be made. The tests of significance in Table II are therefore deduced from the data for 40 to 70°, in

TABLE II

ANALYSIS OF VARIANCE OF TOUGHNESS (RELATIVE WORK DONE
IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS
HEAT TREATMENTS (40 TO 70° C.)

Variance due to	Degrees of freedom	Mean square
Period of heating (average for all temps.)	3	0 77
Error *(a)*	12	1 10
Temperature of heating (average for all periods)	3	3 96*
Interaction, temp. × period	9	3 30**
Error *(b)*	36	1.02

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

which region the factorial combination of treatments was complete.   Table III however includes all of the available observations.

Owing to the fact that the pieces cut from the same back resembled each other more closely than did those from different backs, the comparisons afforded by the experiment are of two levels of precision, as indicated by mean square errors *(a)* and *(b)* in Table II.   Over the temperature range 40 to 70°, the differences between the average toughness after the four periods of heating did not attain statistical significance on the number of observations available. This however was due to a balancing of effects, rather than to an absence of any influence of duration of heating, as, in addition to average temperature effects, a significant interaction of temperature with period of heating is demonstrated in the lower part of this table.

The maximum average toughness, over all four heating periods, was observed after heating at 50° C., but owing to the interaction noted above the maximum for the individual periods varied from 60° for the 5 and 10 hr. periods to 40°

TABLE III

AVERAGE TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO
VARIOUS HEAT TREATMENTS

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40–70°
	20	40	50	60	70	80	
5	3.05	4.05	3.40	4.25	4.28	3.02	3.99
10	3.02	3.75	3.78	4.40	3.02	2.98	3.74
20	3.63	4 35	4.65	3.75	3.48	1.58	4.06
40	—	3.65	5.85	2.78	2.08	—	3.59
Mean	3.31	3.95	4.42	3.79	3.21	2.52	3.84

for 20 hr. and 50° for 40 hr. With the exception of the anomalous value observed after heating at 40° C. for 40 hr., the general tendency was for toughness to increase with duration of heating at temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. It has to be remembered of course that these results were obtained from small pieces of meat, and that the temperature and time effects noted are therefore unlikely to be directly applicable to the commercial treatment of entire sides. Nevertheless, the bearing of these facts upon the operations of commercial smoking will be appreciated, as temperatures ranging from as low as 40° in the smoking of bacon, to 60° or more in the production of "pre-cooked" hams are employed in practice. The reasons, other than possibly the straightforward removal of moisture, for the increase in toughness with time of heating at temperatures up to 50° are at present unknown, but it is of interest to note that the higher temperatures to which "pre-cooked" products are subjected may be expected to have a definitely tenderizing effect.

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FROZEN STORAGE OF POULTRY

IV. FURTHER OBSERVATIONS ON SURFACE DRYING AND PEROXIDE OXYGEN FORMATION[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

A package constructed from moisture resistant material, capable of being ventilated during chilled storage, and sealed to prevent surface drying during frozen storage, is described. Results are presented to demonstrate the ability of this package to maintain the desired humidity conditions. Jacketing a room to separate the cooling coils from the space occupied by the product does not prevent surface drying of boxed goods, presumably because of the absorption of moisture by the boxes. Delays between slaughter and freezing accelerate the development of rancidity in the fat of poultry during subsequent frozen storage, as indicated by the formation of peroxide oxygen. The free fatty acid content is not seriously affected unless the conditions prior to freezing enhance microbial development.

Introduction

It has been shown in earlier papers of this series (1, 2, 3) that surface drying, causing a loss of bloom and development of freezer burn, was the first type of deterioration to occur in poultry during frozen storage. It was also found that conditions favouring surface drying also promoted the development of rancidity in the fat. These results suggested the present studies on methods of packaging for minimizing surface drying, and the effect of delays between slaughter and freezing on the development of rancidity.

The results of previous investigations showed that surface drying could be minimized by lining the boxes with reasonably moisture-resistant stocks, such as waxed paper, provided the folds and joints were adequately sealed. Since adequate sealing of the liners used in wooden poultry boxes is commercially impracticable, other types of packages were studied. One disadvantage of a sealed package is that it maintains a high humidity within the package under all conditions, and during chilled (unfrozen) storage such a condition enhances microbial growth. As a certain proportion of market poultry is held in the chilled state for immediate domestic consumption there is an obvious need for a package that can be readily ventilated or sealed in accordance with trade requirements.

Packaging Experiments

Description of Packages

Initial experiments were undertaken with several standard types of corrugated cartons. Those having telescoping covers proved to be best from the standpoint of packing, storing, and sealing. Commercial trials indicated that the strength of such a package was generally satisfactory for a six-bird size. Standard 12-bird packages were also used experimentally, and the use of heavy corrugated stocks and containers of suitable design would appear to make this size entirely practicable for net weights up to 50 or 60 lb.

Cartons of this type must be rendered moisture-resistant. This was accomplished by various methods, including the application of wax or aluminium foil on one or both sides of the carton. Although the foil has many desirable features, the results of these preliminary tests indicated that the application of a sufficiently heavy coating of wax to produce a glossy surface on the inside was adequate for protecting the product. For commercial use the application of wax to the outer surface would also seem desirable to protect the package against condensate and other contact with moisture. All subsequent tests were conducted with both inside and outside surfaces waxed. No liners were used.

Although the ordinary type of full-telescoping carton was easier to seal than the liners in wooden boxes, it was still found difficult to obtain an effective seal in routine practice. This led to the design of the half-telescoping carton shown in Fig. 1, which proved to be comparatively easy to seal, and also facilitated storage of the product in either a "ventilated" or sealed condition. In this package the full size inner tray fits into another tray of half the height, and is covered by a similar half-height tray. These two cover members meet along the medial line of the container, and can be effectively sealed with a moisture-resistant adhesive tape. This construction also provides a double bottom to strengthen the package. If necessary, the carton can be strapped or wired before storage or transport.

The necessary ventilation during chilled storage was obtained by providing a hole in each end of the inner tray just above the joint of the two outer members. By suitably stamping the telescoping cover opposite these holes it was possible to provide a flap that could be broken open to expose the holes when ventilation was desired, or sealed beneath the tape to provide a moisture-tight package. Previous tests demonstrated that a relative humidity range of 85 to 90% was obtained during chilled storage when the openings in the inner tray were $\frac{1}{2}$, $\frac{3}{4}$, and 1 in. for boxes designed to contain about 25, 40, and 60 lb. of poultry respectively. Liners were not used in these packages since they were unnecessary and might obstruct the openings.

Results

Semi-commercial scale tests were conducted with the new design of package in both chilled and frozen storage. The relative humidity inside the package was taken as the criterion of proper ventilation in the chilled state, while the

TOP TRAY

COVER SHEET
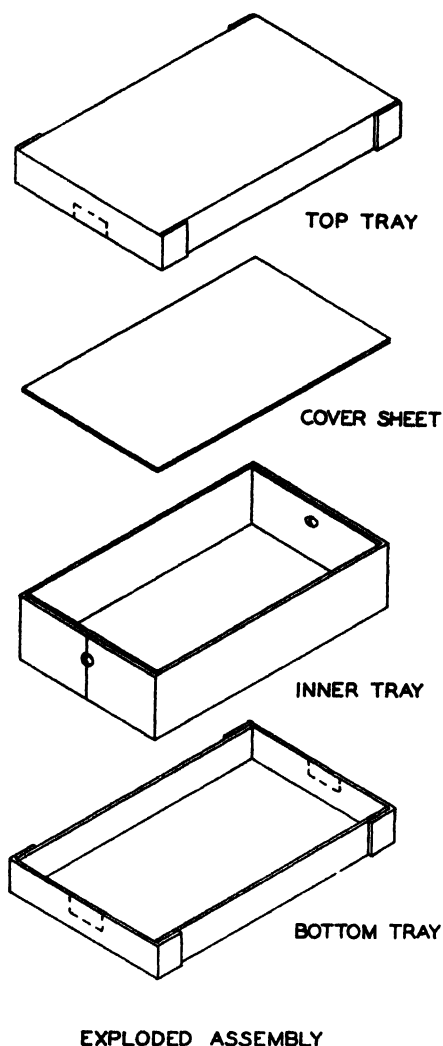
INNER TRAY

BOTTOM TRAY

EXPLODED ASSEMBLY

FIG. 1.   *N.R.C. design of corrugated carton used to facilitate ventilation or sealing.*

proportion of the surface area affected by freezer burn after 27 to 32 weeks' storage at −12° C. was used as a measure of its value for protecting the frozen product against drying.

The chilled storage tests were of 30 days duration at 0° C. During this period two relative humidity measurements were made on each of the experimental boxes using the special hair hygrometer referred to in earlier work (1). Observations were made on both ventilated and sealed packages of the type described earlier, and also on a few of the older types of containers. The results in Table I show that the ventilated N.R.C. type maintained relative humidities between 85 and 90%, which is considered satisfactory. This package in the sealed form, and all the other containers tested, maintained

TABLE I

RELATIVE HUMIDITY IN POULTRY BOXES DURING 30 DAYS' STORAGE AT 0° C.

Type of box	No. boxes in test	Range of size (net wt. poultry, lb.)	Relative humidity inside boxes during storage		
			Max.	Min.	Av.
N.R.C.—ventilated	5	19–75	90	84	86
N.R.C.—sealed	5	22–74	100	98	99
Ordinary telescoping carton—unsealed	1	28	—	—	98
Wooden box with unsealed moisture-resistant liner	2	42–50	98	100	99

humidities approaching saturation, which is clearly too high for satisfactory storage in the chilled state.

The extent of surface drying in the various containers stored in the frozen state is evident from the results presented in Table II. The average value of 2% indicates that the new containers give adequate protection from surface drying. For the most part the small freezer-burnt areas occurred only on certain birds that had been forced in close contact with lightly waxed regions of the cartons. Experienced inspectors examining these boxes reported no evident deterioration, and classed the bloom as "good" or "excellent".

Although only a limited number of other types of boxes were available for comparison, the results are in complete agreement with those obtained in earlier preliminary experiments. An ordinary waxed but unsealed telescoping

TABLE II

SURFACE DRYING DURING STORAGE IN FROZEN STATE

Type of box	No. boxes in test	Storage conditions	Proportion of surface area affected by drying			Remarks
			Max., %	Min., %	Av., %	
N.R.C.—sealed	8	27 weeks at −12° C. 65% R.H.	8	1	2	
N.R.C.—sealed	11	32 weeks at −12° C. 65% R.H.	3	1	2	Figures exclude one damaged box showing 10% F.B.
Ordinary telescoping carton—unsealed	2	32 weeks at −12° C. 65% R.H.	30	3	14	
Wooden box with unsealed moisture-resistant liner	1	32 weeks at −12° C. 65% R.H.	—	—	14	
Wooden box with sealed moisture-resistant liner	2	27 weeks at −12° C. 65% R.H.	3	1	2	

carton, and the usual wooden box with unsealed liner both showed about 14% of the area of the product affected by freezer burn. Inspectors reported deterioration in these boxes. Sealing the waxed paper liner in the wooden boxes reduced the affected area to 2%, in agreement with earlier results (1).

One of the recent developments for preventing drying and shrinkage of individually wrapped birds during storage is the use of thin transparent latex bags. Ten birds were sealed in these bags and exposed, without further protection, for a period of 43 weeks to the conditions described earlier. At the end of the storage period there was no evidence of drying, and the loss in weight was less than 1%.

Experiments in Jacketed Spaces

Primarily, a moisture-resistant packaging provides a vapour barrier between the product and the cooling coils, the regions of maximum and minimum vapour pressure respectively. The introduction of this vapour barrier in the same relative location, but as part of the room rather than as part of the package, might prove equally effective and less costly in the prevention of drying. The use of a jacketed room, as suggested by Huntsman (4), with the cooling coils placed between the jacket and the insulated wall, appears to meet these requirements. Although a cold store of this type would doubtless reduce desiccation, it might not be as effective as moisture-resistant packaging, since there is some evidence (5) that packages such as wooden boxes may themselves absorb considerable moisture from the air at relative humidities approaching saturation. In these circumstances the cooling coils may not be the only agency responsible for drying, and consequently their isolation from the space occupied by the product may be only partially effective in reducing desiccation.

This possibility was examined by placing two boxes of poultry in each of two gas-tight steel tanks to represent jacketed spaces. These were of sufficient size to contain two boxes of poultry in $\frac{2}{3}$ to $\frac{3}{4}$ of their volume. Approximately 20 lb. of ice was placed in the bottom of one of the tanks, in order to provide a source of water vapour, other than the product, for the maintenance of a high relative humidity. The other tank contained only the boxed product. In all instances the poultry was packed in wooden boxes with moisture-resistant unsealed liners. The tanks were stored at a temperature of from -12 to $-15°$ C. for a period of 87 weeks before being opened for examination. This prolonged storage period was used to exaggerate any surface desiccation that might have occurred.

Results showing the condition of the poultry in the two tanks at the end of the storage period are given in Table III. Serious deterioration of the product had occurred in the tank without ice, while that in the tank containing ice did not show marked injury. Although this product was stored for an excessively long period, the results nevertheless demonstrate that the absorption of moisture by the package may cause serious surface desiccation. In these circumstances it is evident that goods packed in containers capable

TABLE III

SURFACE DRYING FOLLOWING 87 WEEKS' STORAGE AT
−12 TO −15° C. IN GAS-TIGHT TANKS WITH
AND WITHOUT ICE

Storage conditions	Surface area affected by desiccation, %	Bloom
In tank without ice	20–25	Poor
	10–15	Poor
In tank with ice	0–5	Good
	0–5	Excellent

of absorbing moisture cannot be stored successfully in a jacketed room unless a high humidity is maintained by some agency other than the stored product.

Formation of Peroxide Oxygen and Free Fatty Acids

The results of a previous investigation on the frozen storage of poultry showed that the fat was relatively resistant to oxidative and hydrolytic changes (3). Although surface desiccation accelerated peroxide oxygen formation, it was found that, even under conditions favouring severe drying, the peroxide oxygen content seldom exceeded 8.0 ml. of 0.002 N sodium thiosulphate after a storage period of 25 months at −13.5° C. It was concluded that the extent of the decomposition of the fat should seldom effect serious deterioration in the flavour of poultry which was promptly precooled and stored at suitable temperatures in the frozen state for normal storage periods.

In commercial practice, delays in cooling or freezing may unavoidably occur, and if sufficiently prolonged may result in a considerable acceleration of the decomposition of the fat. Although such changes may not be evident immediately because of the nature of the induction period characteristic of the development of rancidity, the fat may become rancid quite rapidly during a subsequent period of frozen storage. The material available from the packaging experiments described previously permitted some preliminary observations on this problem.

Peroxide oxygen and free fatty acid determinations were made by methods previously described (3), on the subcutaneous and skin fat of one or two birds taken at random from each of a number of the boxes at the end of the period of frozen storage. This material represented poultry that had been precooled and packed in a commercial plant, and which had been held at temperatures of approximately 0° C. for periods of one week and five to six weeks between slaughter and freezing. Since none of the boxes from which the samples were taken suffered evident deterioration from drying, the accelerating action of surface desiccation on peroxide oxygen formation was excluded.

The peroxide oxygen and free fatty acid contents of the fat and the corresponding storage conditions are shown in Table IV. Poultry held for one week at 0° C., followed by frozen storage at −12° C. for 32 weeks, yielded peroxide oxygen values approximately twice as large as those previously obtained for poultry stored for much longer periods (3). This suggests that delays before precooling or freezing were responsible for the greater deterioration. The free fatty acid contents for this group of poultry were low and approximately normal.

TABLE IV

PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF POULTRY FAT FOLLOWING
VARIOUS STORAGE TREATMENTS

Storage conditions	Peroxide oxygen (as ml. 0.002N Na$_2$S$_2$O$_3$ per gm.)	Free fatty acid as % oleic acid
Precooled commercially, stored one week at 0° C., and 32 weeks at −12° C.	4.0	0.57
	2.7	0.59
	3.0	0.67
	0.8	0.54
	4.9	0.65
	1.7	0.53
Precooled commercially, stored 5 to 6 weeks at 0° C., and 27 weeks at −12° C.	6.3	5.3
	8.8	19.9
	11.1	15.2
	5.1	31.4
	18.4	12.9
Precooled commercially, frozen and stored in a gas-tight tank containing ice for 87 weeks at −12 to −15° C.	9.3	0.95
	6.3	0.60
	6.4	0.88
	11.7	0.89

The next series of samples studied were obtained for poultry stored for five to six weeks at 0° C., followed by storage for 27 weeks at −12° C. Under these conditions the peroxide oxygen values had increased to levels at which the fat would be considered rancid. In addition the free fatty acid content was high, and would undoubtedly exert a deleterious effect on flavour. The excess free fatty acid formation may be attributable to excessive microbial activity during storage at 0° C. These results show conclusively that the product should be precooled and frozen promptly after slaughter in proper storage practice.

It is evident that there is considerable variation in the peroxide content of the fat of birds treated similarly. This indicates a difference in the susceptibility of the fat of different birds to oxidation, and is in agreement with previous findings (3). Doubtless the breeding and feeding of the poultry affects the susceptibility of the fat to oxidation.

The last section of Table IV gives the results of the analysis of the fat of two birds taken from each of the two boxes stored in the gas-tight tank con-

taining ice (see Table III). The peroxide oxygen values were generally higher than those observed previously (3). This may be due to greater delays in precooling and freezing the present material, or to a greater susceptibility of the fats of these birds to oxidation. Nevertheless it is evident that poultry fat may become slightly or definitely rancid under storage conditions that prevent surface drying. Rancidity development is therefore one of the factors limiting the storage life of poultry stored at $-12°$ C. even when surface drying is prevented. These birds were stored for an excessive period, however, and under commercial storage practice, surface drying, which is directly detrimental to quality, and indirectly accelerates oxidative changes, is likely to be the primary factor limiting the storage life.

Acknowledgments

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FREEZER STUDIES—II.

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A study of the improvements in food freezing methods and apparatus during the past 20 years discloses no departure from the fundamental principles embodied in the usual problems of heat exchange as applied to freezer design. The important developments along this line have been confined to advancements in the methods of applying the refrigerating effect for the purpose of accelerating heat exchange during the freezing process, and to improvements in the arrangements of the food freezing and handling facilities.

Among the major limiting factors in freezer design are the freezing capacities required for each kind of food, the maximum freezing time required for some foods, the nature and size of the individually frozen products, initial and operating costs, etc. But other, minor considerations may assume major proportions when they are applied to some specific problem in commercial practice.

An example of one of these minor considerations assuming major proportions is the increased drying that accompanies forced air circulation in the ordinary sharp freezer. Engineers readily appreciate the improvements in heat transfer conditions induced by the use of forced air circulation, but the accompanying increased drying effect in some cases has reached such proportions as to militate against its general use.

The mechanism of drying in a freezer is quite simply explained by differences in water vapour pressures due to differences in temperature. If fish enter a freezer at 32° F. the water vapour pressure at the wet surfaces will be 4.579 millimetres of mercury, and if the cooling coils are at a temperature of 0° F. the vapour pressure there will be 0.956 millimetres of mercury. Naturally the vapour particles free to move will travel from the high to the low pressure points; that is, from the fish to the coils. The vapour particles have inertia and can receive momentum just as can the ubiquitous air particles. So it happens that in stepping up by means of a fan the velocity of air, the main heat carrier in a freezer, the velocity of the moisture particles is inevitably stepped up also; and consequently drying is correspondingly increased. This drying or evaporation requires energy, however, and since the required energy comes from the evaporating surface, that is, the surface of the fish, the temperature at these surfaces is consequently depressed and the vapour pressure correspondingly.

It is seen, then, that though drying in a sharp freezer cannot be avoided, it can be controlled somewhat since it depends on the velocity of the moisture particles, the mean temperature, and temperature differences in the freezer. Furthermore, in the case of whole fish, since the drying takes place from the exposed surfaces, it can be compensated for in the subsequent glazing operation. Consequently the upper limit of drying may be set at something just below the point where the drying becomes noticeable and detracts from the appearance and therefore the saleability of the produce. That is to say, for given temperature conditions in any freezer there will be a critical rate of air and vapour movement above which damage will occur to the product in the way of noticeable
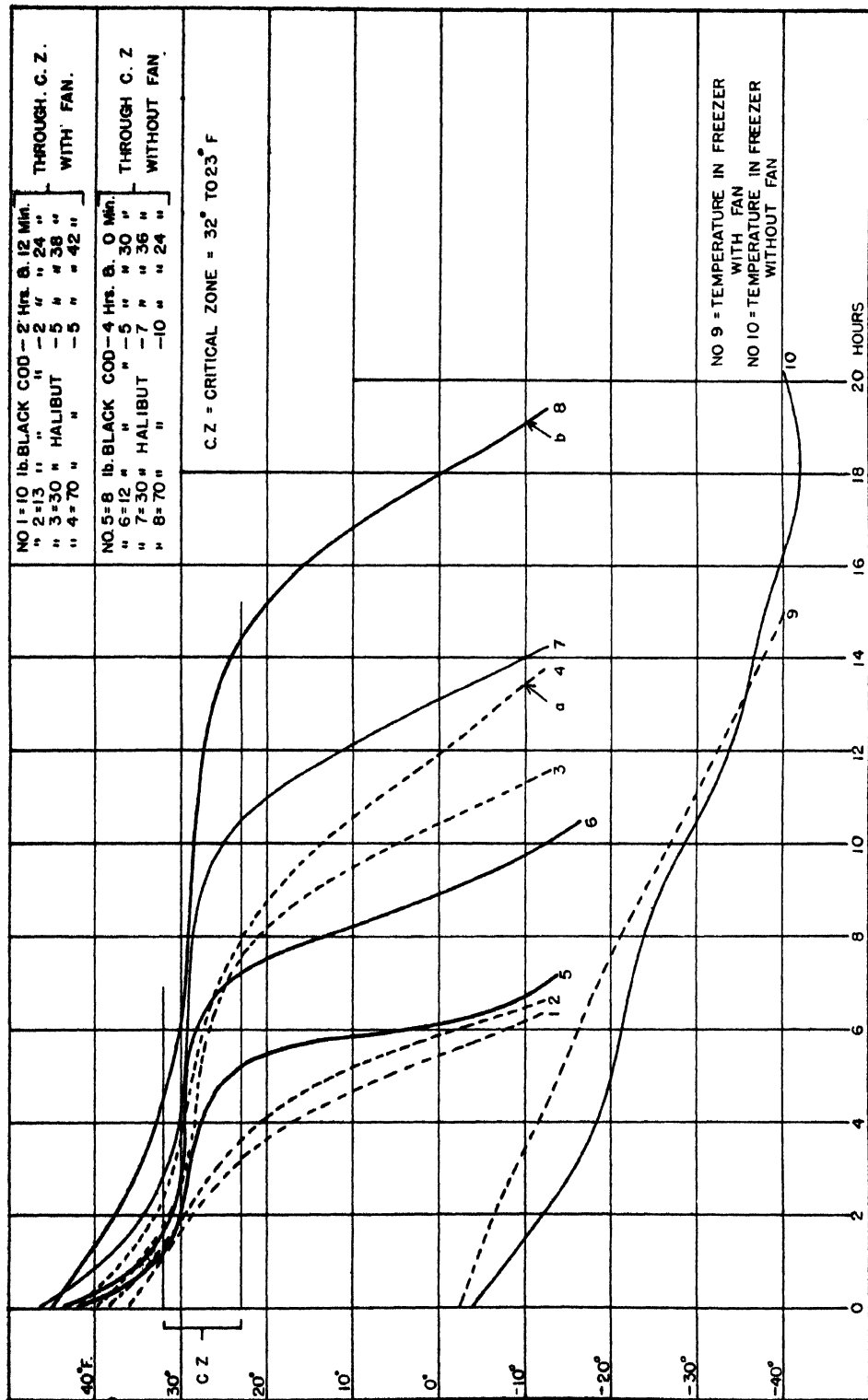
drying. Under favorable temperature conditions this critical velocity will be high and the consequent increase in freezing rate will be commercially important, while under adverse conditions this critical velocity will be so low that it will have no significant effect on the freezing rate.
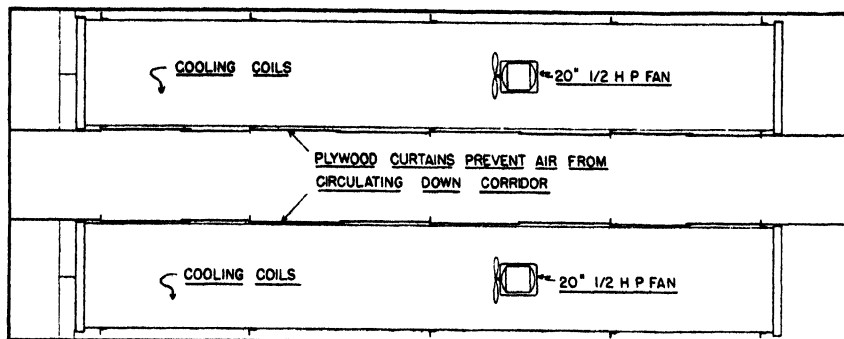
The conditions that permit increased air circulation in a sharp freezer without excessive drying are a low initial temperature with ample cooling-coil surfaces, ample compressor capacity to depress the temperature in spite of the addition of unfrozen fish, and an even temperature distribution throughout the freezer, so that all fish in the freezer will be subjected to the same temperature conditions. To meet these demands requires some modifications to the usual designs and practices, and to obtain results of practical significance requires a full-scale application. An opportunity to make full scale experiments was afforded by the recent extension of refrigeration operations by the British Columbia Packers Limited, who kindly placed at our disposal facilities for certain investigations.

Figure 1 shows the plan and elevation of the experimental freezer installed in this company's plant at Namu, B.C. The cooling system was of the flooded type with float control, and to produce the desired low temperatures double stage compression by means of a booster compressor was used. The modifications consisted of a plywood enclosure across the front of each side of the freezer, shutting off the corridor completely. Since no cooling takes place in the corridor, it is economical to have the air quiescent there. That portion of the enclosure covering the front of the coils where the fish are loaded was made of large sheets of plywood fitted with metal hooks to engage the top outside pipe of the coils. These sheets were thus removeable; but when in place, confined the air circulation within the coil space. A 20-inch, 4-bladed fan was installed for these experiments in the enclosure or duct above one set of coils only, and was placed to force the air longitudinally down the duct. The metal trays on each bank of coils, together with the plywood curtains, formed ducts through which the air was forced in order to get to the suction side of the fan again. The air intake ends of these banks or ducts, indicated by arrows in the elevation in figure 1, were fitted with plywood dampers in order that the air flow could be equalized in the various shelves, or could be shut off entirely should the freezers be only partly filled, or should the fish on some shelves become frozen sooner than on others due to differences in size or kind of fish. Since there was no cooling in the upper enclosure in which the fan was situated, and since the fish were placed directly on the metal trays on the coils in the usual way, the air circulating down the ducts in which the fish were placed was simultaneously heated by the fish and cooled by the coils. Consequently all fish would be under as like conditions as possible. Different air velocities were obtained by means of a V-belt and cone pulleys on a constant-speed motor and a countershaft to which the air propellor was attached, the object being to determine the highest air velocity permissible without noticeable drying under the specific conditions in the freezer.
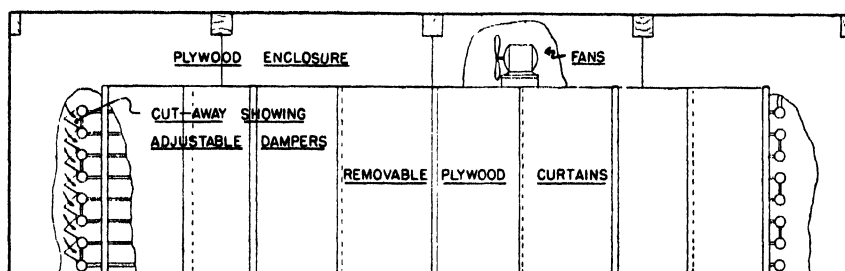
Cooling curves were obtained for the mid-point of different fish in the experimental freezer and in a control freezer which was identical with the experimental one except for the enclosures and the fan. For the various tests both freezers were loaded at the same time, to the same extent and in a similar manner, to facilitate making comparisons. Temperatures were taken by means of thermocouples of the skewer type inserted in tubes penetrating the fish obliquely in the plane of the major axis to minimize heat conduction along the tube.

Figure 2.

COOLING COILS

20" 1/2 H P FAN

PLYWOOD CURTAINS PREVENT AIR FROM
CIRCULATING DOWN CORRIDOR

COOLING COILS

20" 1/2 H P FAN

PLAN OF EXPERIMENTAL FREEZER

PLYWOOD ENCLOSURE

FANS

CUT-AWAY SHOWING
ADJUSTABLE DAMPERS

REMOVABLE PLYWOOD CURTAINS

ELEVATION

Figure 1.

The results of a typical test, when both freezers were approximately three-quarters full of black cod along with a few hundred pounds of halibut of various sizes, are shown in figure 2. The air velocities in this test varied from approximately 200 f.p.m. (feet per minute) over the smaller fish on the top shelves, to 365 f.p.m. over the larger fish at the bottom, and the dampers were kept fixed throughout the test to simplify the interpretation of the data. In practice, however, as soon as the fish on a particular bank of coils become sufficiently "frozen" the damper to that bank would be closed to cut down drying there and to speed up freezing on the other shelves. Fish may be assumed to be sufficiently "frozen" when the temperature at their mid-point has reached the temperature at which they are to be stored, which in these experiments was -10° F.

It will be noticed that in the ordinary or control freezer it took 10 hours 24 minutes to reduce the temperature at the centre of a 70-pound halibut (curve 8) through the critical zone (C.Z.), i.e., from 32°F. to 23°F.; while in the experimental freezer it took only 5 hours 42 minutes for the same sized fish (curve 4). This constitutes a reduction of approximately 45% in time. However, to lower the temperature in these fish from their initial temperature to -10° F. required 18⅓ hours and 13½ hours respectively; therefore a reduction of only about 26% was experienced in the time for the complete freezing operation. After the fish were taken from the freezers they were critically examined by graders and packers, who were unable to pick out those fish frozen in the experimental freezer from the control fish. Therefore the drying was not excessive even in the smaller fish which were subjected to the action of the fan for the full time required to freeze the larger fish. It was assumed, therefore, that for the specific conditions encountered in this test the fan speed employed could be safely used, and that the safety factor would be increased relative to the smaller fish if the dampers to their shelves were closed as soon as the fish reached the desired low temperature.

Referring still to figure 2, it will be observed that the temperature in the experimental freezer was always above that of the control freezer until the fish in the former were about all frozen. This signifies that the back pressure was higher and that the compressors could operate at a more economical level on this freezer.

In all, six tests were conducted with the experimental freezer at Namu. The air velocities were varied, different kinds and sizes of fish were tested, and different temperature conditions were encountered from day to day depending upon the amounts and kinds of fish handled, so that a good deal of interesting and important information was obtained. Time has not permitted the complete analysis of these data, which will be included in a future report along with the results of further studies in this connection.

The use of a fan in these experiments proved exceedingly efficacious; indeed, the design of the entire freezer has considerable merit. However it must not be assumed from these results that fans may be used indiscriminately in freezers. They may only be used to advantage in freezers that have efficient and capacious cooling coils, and where temperatures are kept sufficiently low. Also, the permissible velocity of the air will depend upon the low temperatures obtainable. Although the experimental freezer as shown in figure 1 proved very satisfactory, many improvements suggested themselves for investigation in further studies.

COLOUR OF MEAT

III. AN IMPROVED COLOUR COMPARATOR FOR SOLIDS[1]

BY C. A. WINKLER[2], W. H. COOK[3], AND E. A. ROOKE[4]

Abstract

A photoelectric colour comparator, previously described (Can. J. Research, D, 17 : 1-7. 1939) has been improved to permit greater precision and more rapid operation. Measurements on meat indicate that the degree of precision attainable with the new comparator is determined primarily by sampling, rather than instrumental, errors.

Introduction

An objective colour comparator suitable for estimating the colour of solids was described in the first paper of this series (1). The results obtained with this instrument in subsequent investigations on the colour (2, 4) and colour stability of bacon (3, 5) led to the construction of an improved model. Enquiries requesting a more detailed description and other information regarding the earlier apparatus prompted the preparation of this article describing the new instrument.

The new apparatus, like the old, is based on the measurement of the amount of each main component of white light, namely, blue, green, and red, scattered by the test sample and expressed as a percentage of that scattered by a standard white surface. Such measurements naturally yield less information than those made with a spectrophotometer, but have the advantage that they can be made more rapidly. This feature enables measurements to be made on labile materials without appreciable change, and facilitates making a sufficient number of measurements to estimate the variability present in such biological materials as meat. The equipment was originally designed to place the subjective visual estimates of colour and colour stability on an objective basis. Subsequent papers (3, 4, 5) have shown that not only was the older instrument capable of detecting significant differences in the colour of different samples, but it was also possible, by statistical studies of a large number of measurements, to relate the scatter of the individual colour components to the composition of the bacon with respect to certain constituents.

Description of Apparatus

The new instrument is fundamentally the same as the earlier model. The most important changes are: baffle plates to minimize internal reflections; diaphragm for controlling light intensity, thus enabling the light source to be

operated at fixed voltage and avoiding major change in colour quality; a more sensitive photo-cell, and a more sensitive galvanometer.   Mechanically the apparatus was much improved, permitting greater accuracy and more rapid operation.

A photograph of the apparatus is shown in Fig. 1 and a diagrammatic sketch in Fig. 2.   It is impossible to give complete details of construction in this article but the information contained in the figures and the following brief description are considered adequate to permit the construction of a comparable instrument wherever the necessary facilities are available.

FIG. 1.   *Colour comparator showing general arrangement, lamp housing, reference and test sample holders, and selective filter holder.*

The two sections of the lamp housing, A, and the adapter section, K, are dural castings with drilled and tapped flanges for attachment.   The remainder of the apparatus is constructed from flat or angle aluminium fastened with screws to facilitate assembly.

The two sections of the lamp housing are water jacketed, and a continuous circulation of cooling water is maintained during operation.   The hemi-spherical portion at the back is polished on the inside.   A semi-circular opening is provided between the two halves of the lamp housing to accommodate a No. 2 photo-flood bulb.   The walls of this portion of the housing are thicker and shaped to permit the attachment of an aluminium shield, supporting the lamp socket, to the housing with screws.   As the photo-flood bulb must be screwed into the socket from the inside, the two sections of the housing must be separated when the bulb is changed, but as this is seldom necessary it is not a troublesome operation.
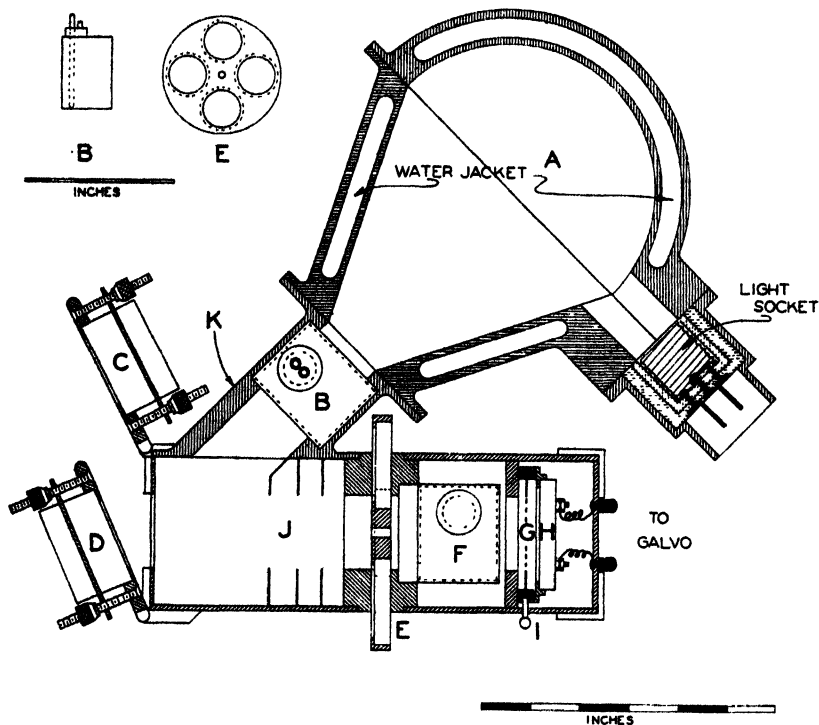
FIG. 2. *Diagrammatic sketch of colour comparator (plan view).*

The adapter section, *K*, is of rectangular cross section and flanged for attachment to the lamp housing. The end furthest removed from the housing is shaped to permit attachment to the metering portion at an angle of 45°. An oval opening, 1.5 in. high and 1.06 in. wide, is provided through the centre of this block, to allow the incident light at an angle of 45° to fall uniformly on the 1½ in. diameter circular opening containing the test sample. A rectangular recess, open at the top, is provided at right angles to the path of the light to accommodate the water cell, *B*, for removing part of the infra-red radiation from the incident light beam. This cell may be glass but a plastic (plexiglass) has been found more convenient. The cap for this cell is provided with two tubes for the circulation of cooling water (tap) during the course of the measurements.

The reference standard and test sample holders, *C* and *D*, consist of removable doors hinged on opposite sides of the rectangular part of the instrument. Each door consists of two plates arranged to hold the sample between them by clamping. The outer plate is solid, lightproof, and moves back or forth on the clamping screws. The inner plate is fixed and has a centrally drilled hole 1½ in. in diameter to expose the material. A similarly drilled plate member, darkened on the inside, forms the back of the instrument. The test sample is usually placed on an aluminium plate, or in a short cylinder with a locating pin if the exact test position is important, and covered with

a clean glass slide before clamping in the holder. A similarly prepared cell containing magnesium carbonate is used as a reference standard.

Light scattered by the sample passes the baffles, *J*, and falls on a glass filter held in a rotating stainless steel holder, *E*, fitted in a light-tight manner into a slot in an aluminium block. Stainless steel was found to produce less friction and stand up better to the wear imposed on this member than an aluminium holder. Following the selective filters is a cell containing a 1% copper sulphate solution. This cell is identical with the water cell except that it has an ordinary screw cap since openings for water circulation are unnecessary. The iris diaphragm, *G*, is placed immediately in front of the photo-cell, *H*, and is adjustable by a short lever, *I*, at the side of the instrument. The centre inner surface between the sample holders and the photo-cell must be light-tight and finished with a flat black paint.

Accessory Equipment

The following accessory parts, obtainable from the stated sources, have been found suitable after considerable experimentation.

Plexiglass cells—made to dimensions and specifications by Stricker-Brunhuber Corp., New York, New York.

Selective filters—

Blue (400–500) Jena BG12, or Corning Signal Blue No. 556.

Green (500–600) Wratten W58, or a combination of Corning Light Theatre Blue No. 502 and H. R. Noviol No. 352.

Red (600–750) Jena OG3 or Corning H. R. Lantern Red No. 244.

Iris diaphragm—Leitz catalogue No. 8503.

Photo-cell—Visitron F-2A. G.-M. Laboratories Inc., Chicago.

Galvanometer—Box type Catalogue No. 4625, Rubicon Co., Philadelphia.

Transformers—The Variac transformer, General Radio Co., Cambridge, Mass., used for controlling the light source is generally unnecessary if an iris diaphragm is used, but may be required in special circumstances. Where line voltage is subject to some variation a constant voltage transformer will improve the accuracy and facilitate rapid operation. Suitable types are available from the Sola Electric Co., Chicago.

Operation

The manipulation of the new machine is simple and rapid. The filter holder is rotated until the desired filter is in position, the standard white holder swung into place, and the galvanometer deflection adjusted to the desired value with the diaphragm. The standard white holder is then swung out, the test sample swung into position and the galvanometer deflection observed. This sequence of operations can be performed in less than a minute for each colour filter used. In fact, with reasonably constant voltage, the three colour components can be measured in two minutes, including the

time required to place the test piece in the holder and remove it. This is a much more rapid rate than attainable with the older instrument and greatly facilitates the measurement of colour in routine operations such as process control. The apparatus is not only suitable for measuring the colour of such solids as meat, but has also been applied to ground materials, such as flour.

Accuracy

The accuracy of the new model can be compared with that of the old model, or with the error of sampling such biological materials as meat, from the results presented in Table I. The mean values and the standard errors of a single measurement were computed from duplicate measurements on some 30 to 40 samples picked at random from routine observations. The measurements on bacon with the old and new models were not made on the same samples and this may account for a portion of the difference between the mean values obtained with the two instruments. The greater part of this difference, however, is attributable to the leakage of light in the older model. The "dark" constant obtained with a standard black, as a test sample, was about 22% of that obtained from the standard white in the earlier model, and only 4% in the new model. This reduction in the dark constant was brought about largely by the insertion of the baffle plates, *J*, in the new instrument. The difference of 18% accounts for most of the difference between the corresponding means reported in Table I.

TABLE I

PRECISION OF NEW COLOUR COMPARATOR AS COMPARED WITH THAT OF OLDER INSTRUMENT
AND SAMPLING ERROR

Colour component	Old instrument		New instrument				
	Bacon only		Bacon		Pork		
	Mean	Std. error (instrument)	Mean	Std. error (instrument)	Mean	Std. error (sampling)	Std. error (instrument)
Initial colour							
Red	43 2	1.41	25 4	1 00	25.7	1 48	1.13
Green	31.1	0.87	14 5	0 45	16 2	1 12	0.77
Blue	28.3	0 61	11 5	0 36	11 9	0 74	0.64
Brightness					53.9	3.37	2.29
*Colour stability**							
Red	5.35	1.30	4.03	0 60	2.19	0.43	1.04
Green	3.66	1 03	2 70	0.50	0.77	0.53	0.92
Blue	2.57	0 89	2 50	0.40	0.87	0 37	0.63
Brightness					3.83	1.33	2.22

Mean change of scatter, usually a decrease from the initial value.

The standard error of the instrument was computed by statistical methods from the difference between duplicate observations. The two values were obtained by cutting a piece of meat and making a single measurement on each of the two surfaces so produced. This practice tends to exaggerate the instrumental error by including the error of subsampling the test surface, and duplicate measurements on the same surface can be checked within narrower limits. The results of measurements of both the initial colour and colour stability of bacon show that the standard error of the new instrument is about half of that observed on the old instrument.

When the standard error of the initial colour measurement is expressed as a percentage of the mean for the corresponding colour component, the accuracy varies from about ±2 to 4%. This may appear to represent satisfactory precision, but it must be remembered that a difference of 10 to 20% in scatter represents the difference between a satisfactory and wholly unacceptable colour as judged by visual standards. In these circumstances it might appear that a still higher instrumental precision would be desirable for making fine distinctions, without the need for tedious replication.

In connection with an investigation into the storage of pork, provision was made for estimating both the sampling and instrumental errors by making duplicate measurements on duplicate pieces of pork taken from the same part of the same carcass and treated identically throughout. An analysis of variance showed that the combined sampling and instrumental errors were significantly greater than the instrumental error alone. It appears therefore that the sampling error is the source of variation limiting the accuracy of initial colour measurements and that little, if any, increase in precision would be accomplished by further refinements in the instrument.

These results were analyzed further with the object of determining the method of measurement capable of yielding the greatest precision for a limited number of observations. The last two columns show the standard error of sampling and instrumental measurements independently, on a single observation basis. The sampling error for the initial colour estimations is in all instances larger than the instrumental error. It is therefore evident that four single instrumental observations on four separate samples taken from the test material would yield a more accurate estimate of the true colour than duplicate instrumental readings on two samples. In other words, the instrumental error is not the factor limiting the accuracy of colour measurements on meat.

A somewhat different situation exists with respect to the error of estimating the change in colour during colour stability measurements (lower section, Table I). Although the standard instrumental error is of the same order as that for the initial colour measurement, the change in colour scatter is much smaller than the original scatter and is consequently estimated with much less precision on a percentage basis. As can be seen from Table I, the sampling error of colour stability measurements is less than the instru-

mental error. In these circumstances precise estimates of colour stability can be obtained with the present equipment only by adequate replication.

From the standpoint of practical colour measurements on a particular material, meat, flour, etc., the most important factor is to select colour filters that will yield the maximum information on colour quality for the material in question. In this respect the new instrument is the same as the old, since the filters separating the three broad colour bands are the same. Spectroscopic studies of meat are now under way with the object of determining the practicability of using more selective filters. Should such a modification be desirable, it is possible that the use of extremely narrow colour bands might require more sensitive measuring equipment.

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CANADIAN WILTSHIRE BACON

XIV. SEASONAL VARIATIONS IN COLOUR AND COLOUR STABILITY[1]

By C. A. Winkler,[2] W. H. Cook,[3] E. A. Rooke,[4] and A. E. Chadderton[4]

Abstract

Measurements of the colour and colour stability of Wiltshire bacon, cured in a factory known to produce a generally satisfactory product, showed that there were small but significant differences in these properties from time to time, but there was no definite evidence to indicate that these differences could be attributed to systematic seasonal effects.

Introduction

Colour and colour stability are two important attributes of quality in bacon. Previous investigations (3, 4) on this subject have shown that, although Wiltshire sides cured at the same time in the same factory, may differ significantly in colour quality, the main source of variation generally lies in curing sides in different factories. This suggests that the colour quality of bacon depends primarily on the handling and curing practices followed in different establishments. There is also the possibility that colour quality may show seasonal variations, due either to the influence of environment on the animal prior to slaughter or to the effect of minor variations in the handling or curing conditions at certain stages where all the conditions are not subject to close control. The observations of certain practical operators suggested the existence of such seasonal variation in colour. The present study was undertaken to examine this possibility.

Material and Method

All the samples were obtained from a plant known to adhere closely to a practice that produced sides of consistently satisfactory colour quality. One side was taken at random from each weekly batch over a period of 20 months. After maturing at 32 to 35° F. for two weeks after removal from the curing tank, a portion, always taken from the same position in the prime back, was examined in the pale or unsmoked state.

The colour measurements were made with the colour comparators described in earlier publications. The first instrument (1) was used for the early measurements, and the improved form (2) during the remainder of the period. Owing to the higher "dark" constant of the first instrument, the readings so obtained were greater than those observed with the improved

model. For this reason the results with the two instruments must be considered separately.

Estimates of colour stability were obtained from the change in colour during exposure for 24 hr. at 10° C. and 60 to 70% relative humidity. This relative humidity is lower than that employed in earlier measurements, and was chosen to include the effect of both drying and oxidation, comparable with that occurring in retail stores.

It has been shown in earlier papers (3, 4) that there is some variation between the colour and colour stability of different sides cured in the same tank at the same time. This inherent difference between sides must be considered in estimating the significance of differences that might otherwise be attributed to seasonal or time effects. Since it was impossible to provide samples from two sides, each week, it was assumed that the difference observed over a two-week period (three observations: beginning, middle, and end of period) would unlikely be seasonal, and consequently could be taken to represent the difference between sides. The observed differences were therefore analysed into portions attributable to within and between these two-weekly periods, taken as representing the difference between sides and seasonal effects respectively.

Results

The results of these analyses of variance appear in Table I. They show that on the average the differences between two-week periods, indicative of time effects, were usually significant for both the initial colour and colour stability measurements made with both instruments.

Figs. 1 and 2 were constructed from the results of the colour and colour stability measurements in an attempt to determine whether the significant differences, demonstrated between two-week periods, could be attributed to seasonal effects. Each point represents the mean of the three observations taken during each period. The central horizontal line indicates the general mean for all measurements made with the given instrument. The cross-hatched section on each side of the general mean indicates the necessary difference, computed from the variance within periods, between the individual points and the general mean.

It is evident from Fig. 1 that the initial colour during a given period seldom exceeded the necessary difference. The measurements made during June, 1938, with the original instrument were generally significantly below average, and increased to somewhat above average during August, 1938. Subsequent variations did not attain significance. Since the below- and above-average values observed in 1938 did not appear in 1939 when measurements were made with the new comparator there is no evidence to indicate that the difference between periods are attributable to systematic seasonal effects.

Fig. 2 shows the results of the colour stability measurements. The amount of light scattered by bacon generally decreases during exposure, and this was particularly true in these tests conducted under conditions that allowed

TABLE I

RESULTS OF ANALYSES OF VARIANCE ON COLOUR AND COLOUR STABILITY MEASUREMENTS

Source of variance	Degrees freedom	Mean square			Total brightness
		Red	Green	Blue	
Initial colour of internal surface (original instrument)					
Between two-week periods	17	40.28**	36.30**	35.68**	293.05**
Within two-week periods (Difference between sides)	36	8.29	5.24	2.84	42.40
Initial colour of internal surface (new instrument)					
Between periods	9	19.50*	6.90	4.04*	81.02*
Within periods	20	7.34	2.90	1.59	30.86
Colour stability (original instrument)					
Between periods	17	20.51**	3.89**	18.56**	99.94**
Within periods	36	3.84	1.02	2.58	15.32
Colour stability (new instrument)					
Between periods	9	3.48*	1.70**	0.69*	14.97**
Within periods	20	1.20	0.46	0.26	3.75

Indicates 5% level of significance.
**Indicates 1% level of significance.*

some drying to occur. In consequence, the initial value minus the final value is positive. This difference is used as the ordinate in the figure. Since the points are all greater than zero some darkening is indicated for all samples. A marked darkening, or instability, occurred during August, 1938, when the colours of the original samples were brighter than average. Otherwise none of the points show a significant departure from the average of all observations made with the original instrument. During August, 1939, the bacon, measured with the new instrument, showed greater stability than the average. This appears to be associated with the darker samples (Fig. 1) obtained at that time. From these results it appears that, although colour stability may be associated with the initial colour of the samples, there is no evidence of definite seasonal trends.

Discussion

Attempts to relate the significant departures from average colour quality to the use of frozen carcasses and to other minor changes in curing practice indicated that these were not the causative factors. Likewise the temperature records for the years 1938 and 1939 failed to show anything that would explain the variations. The significant departures from the average, therefore, remain unexplained.
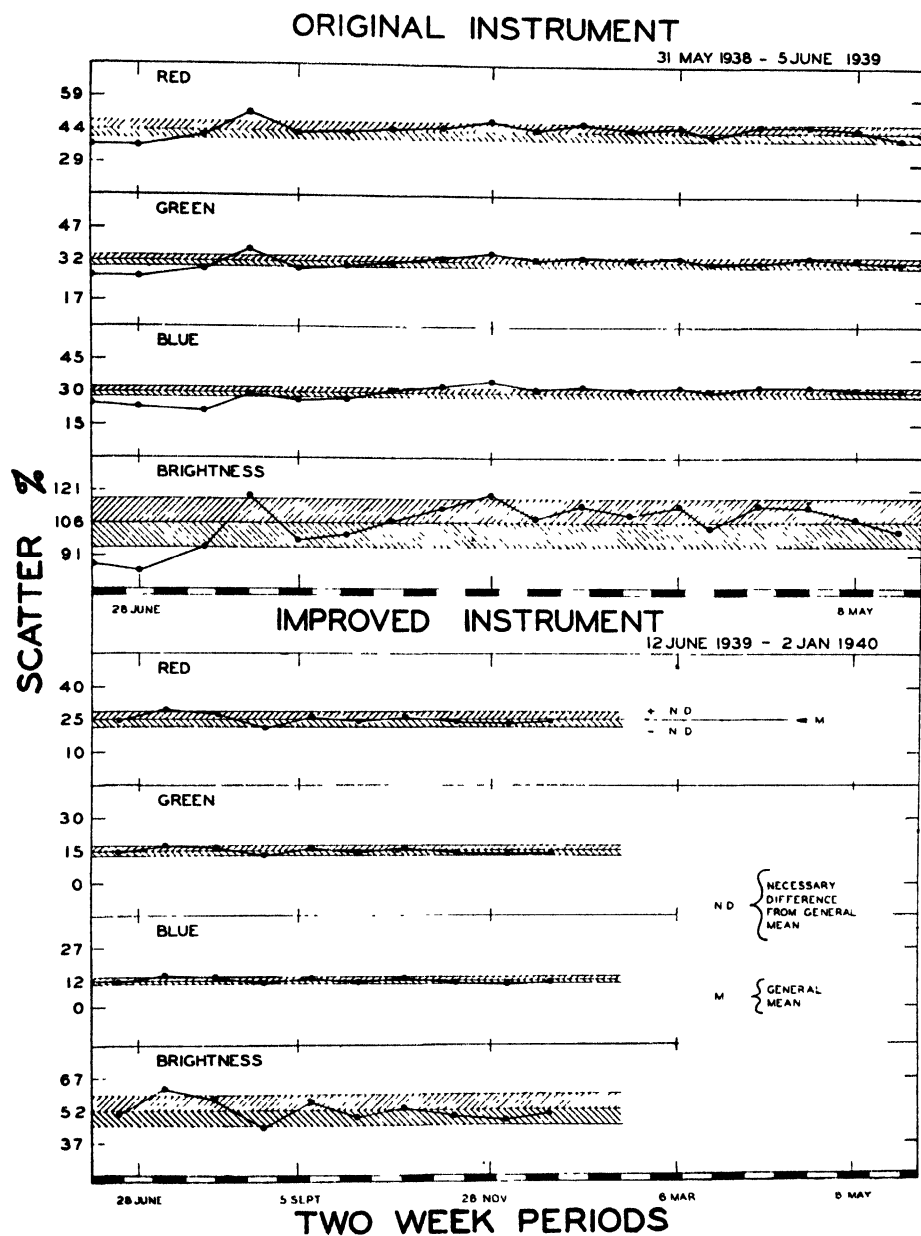
ORIGINAL INSTRUMENT

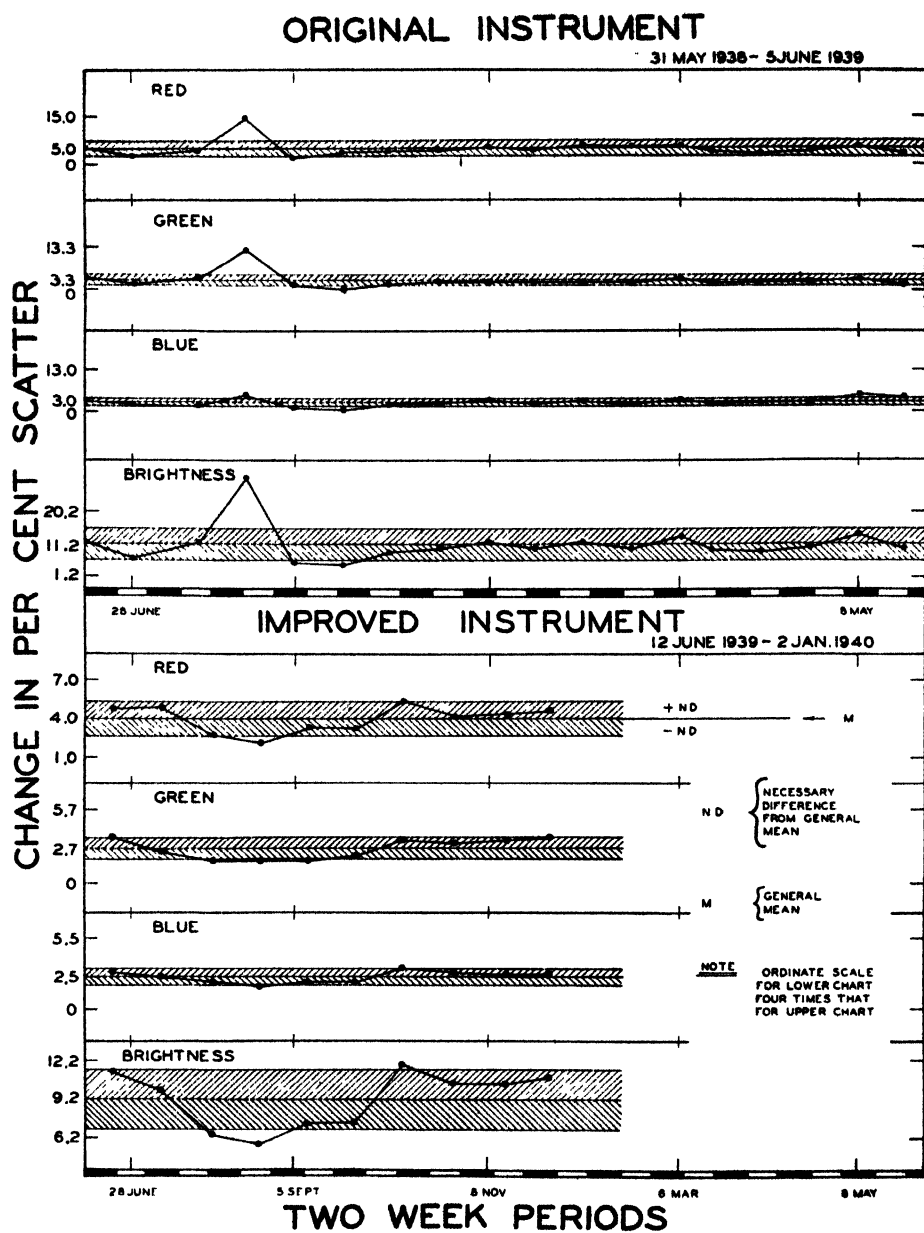FIG. 1. *Seasonal variations in colour scatter.*

FIG. 2. *Seasonal variations in colour stability*

It is entirely probable that the curing and handling practices followed in certain factories may enhance possible seasonal variations in colour quality. The results of the present investigation, however, indicate that practices could be adopted that would render the product relatively independent of these effects, but the nature of the detrimental practices, if any, must await the results of future investigations.

References

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